Committee for Risk Assessment

RAC

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

20 September 2019

CLH-O-0000006712-75-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical 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)**

The proposal was submitted by **The Netherlands** and received by RAC on **15 August 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **29 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 January 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **20 September 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	614-RST-VW-Y	emamectin benzoate (ISO); (4''R)-4''-deoxy-4''-(methylamino) avermectin B1 benzoate	-	155569-91-8	Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 STOT RE 1 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H311 H301 H372 (nervous system) H318 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H331 H311 H301 H372 (nervous system) H318 H410		inhalation: ATE = 0.663 mg/l dermal: ATE = 500 mg/kg bw oral: ATE = 60 mg/kg bw M=10000 M=10000	
RAC opinion	614-RST-VW-Y	emamectin benzoate (ISO); (4''R)-4''-deoxy-4''-(methylamino) avermectin B1 benzoate	-	155569-91-8	Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 STOT SE 1 STOT RE 1 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H311 H301 H370 (nervous system) H372 (nervous system) H318 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H331 H311 H301 H370 (nervous system) H372 (nervous system) H318 H410		inhalation: ATE = 0.663 mg/l (dusts or mists) dermal: ATE = 300 mg/kg bw oral: ATE = 60 mg/kg bw STOT RE 1; H372: C ≥ 5 %; STOT RE 2; H373: 0,5 % ≤ C < 5 % M=10000 M=10000	

Resulting Annex VI entry if agreed by COM	614-RST-VW-Y	emamectin benzoate (ISO); (4''R)-4''-deoxy-4''-(methylamino) avermectin B1 benzoate	-	155569-91-8	Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 STOT SE 1 STOT RE 1 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H311 H301 H370 (nervous system) H372 (nervous system) H318 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H331 H311 H301 H370 (nervous system) H372 (nervous system) H318 H410		inhalation: ATE = 0.663 mg/l (dusts or mists) dermal: ATE = 300 mg/kg bw oral: ATE = 60 mg/kg bw STOT RE 1; H372: C ≥ 5 %; STOT RE 2; H373: 0,5 % ≤ C < 5 % M=10000 M=10000	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Emamectin benzoate (ISO) has no Annex VI in CLP, and it is not registered under REACH (July 2019). It consists of two structurally complex heterocyclic compounds with a minimum of 920 g/kg of emamectin B1a benzoate and a maximum of 50 g/kg emamectin B1b benzoate.

Emamectin (base) has been evaluated in the context of Regulation EC 1107/2009 (EFSA, 2012). The CLH report submitted by the Dossier Submitter (DS) is based on the plant protection draft assessment report (DAR, 2011) and it contains data on several forms of emamectin (e.g. emamectin hydrochloride and emamectin benzoate hydrate). These forms are toxicologically equivalent, the emamectin moiety being considered as the active toxicophore. The DS noted that most of the studies used dose levels recalculated as emamectin (free) base compound to account for differences in the molecular weights of the salts. However, since this CLH proposal concerns emamectin benzoate (ISO), the DS applied corrective factors to express doses as emamectin benzoate equivalents. Conversion factors are presented in the Annex 1 to the CLH report and in the Competent Authority Report (CAR). RAC notes that some dose levels remained expressed as emamectin base compound in the CLH report and this has been taken into account in the assessment when possible.

RAC notes that 0.76 % w/w propyl gallate was added as an antioxidant in some studies, presumably to prevent the auto-oxidation or decomposition of the active substance. However, this was not discussed in the study reports and the DS has not assessed the potential impact, if any, of propyl gallate on the study results. RAC is of the opinion that propyl gallate, which is recognised as an acceptable excipient in pharmaceutical products and as a food preservative and antioxidant for animal fats and oils, has probably very limited influence on the toxicity of emamectin benzoate, since similar results were obtained from studies without propyl gallate. Indeed, it is likely that in the event toxicity is influenced that it would favour a reduction in adverse effects, but this is beyond the remit of this particular assessment. Therefore, RAC finds no reason to exclude these studies on emamectin benzoate for the purpose of classification.

Modes of action

The modes of action of the effects of emamectin benzoate on the nervous system are not fully established. The recent scientific literature has suggested different mechanisms, including a pharmacological action common to all avermectins (abamectin, ivermectin, emamectin) via the interaction with the gamma-aminobutyric acid (GABA)-benzodiazepine receptor channel complex. Avermectins increase the membrane permeability to chloride ions in nerves and muscle membranes and act as GABA agonists. In mammals, GABA-containing neurons and receptors are found in the central nervous system (CNS) i.e. the brain and the spinal cord), but not in the peripheral nervous system. GABA plays a critical role in nervous system development through both non-synaptic and synaptic mechanisms. Consequently, emamectin benzoate may have the potential to influence GABA-mediated events important for brain development as well as influence GABA-mediated regulation of metabolism, food intake and body weight, as observed in some toxicological studies. Although GABA receptor mediated neurotoxicity is a well-known phenomenon, the adverse outcome pathway for this effect has not been fully established.

Several avermectins have also been shown to interact with the adenosine triphosphate-binding cassette (ABC) transporter p-glycoprotein. The ABC transporter P-glycoprotein is widely distributed in tissues, but importantly, in the present context, it is expressed in capillary endothelial cells constituting the blood-brain barrier. It is involved in the transmembrane transport of various molecules, and it functions to remove toxic substances from the brain.

Mutant CF-1 mice deficient in expression of mdr1a P-glycoprotein show a much higher sensitivity to neurotoxicity of emamectin. In addition, neonatal rats are known to have a limited expression of P-glycoprotein until about 5 weeks of age and, of equal if not more importance, an incomplete development of the blood-brain barrier both before and after birth (Lankas *et al*., 1989, Matsuoka *et al.,* 1999; Betz and Goldstein, 1981; JMPR, 2011; EFSA, 2007; EFSA, 2012).

Consistent with the above modes of action, the main target organ for emamectin is the nervous system. However, RAC considers that, in line with the RAC (2010) opinion for abamectin (ISO) and EFSA (2012), CF-1 mice do not constitute a good model for assessing the neurotoxicity of emamectin benzoate. RAC notes the difficulty of assessing data of neurotoxic substances with regard to the susceptibility of the neonatal rat vs. the potential susceptibility to the human neonate.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Emamectin benzoate is a white solid at room temperature. The DS concluded that some hazard classes were not applicable (e.g. gas or liquid related physical hazards) or proposed no classification due to lack of data (pyrophoric solid, substance corrosive to metals, substance which in contact with water emits flammable gases and for a self-reactive substance). The DS also considered emamectin benzoate (ISO) not self-reactive based on the absence of N-oxides (Section 2.8.4.2 of the CLP guidance, version 5, July 2017) and not pyrophoric or capable of emitting flammable gases based on handling experience.

For the three remaining physical hazards, the DS proposed no classification based on the following data:

- Flammable solids: a test conducted according to method A.10 (Angly, 2000a) gave a result "not highly flammable".
- Self-heating substance: the onset temperature for self-heating of emamectin benzoate started at 395 °C, which is above the classification limit of > 140 °C (Angly, 2000b).
- Explosive properties: a negative test conducted according to method A.14 (Angly, 2000c) supported by the absence of any chemical groups associated with explosive properties (Section 2.1.4.2 of the CLP Guidance).

Comments received during the consultation

No comments were received during the consultation.

Assessment and comparison with the classification criteria

RAC agrees with the DS on no classification for hazard classes applicable to gases or liquids since emamectin benzoate is a solid at room temperature. RAC also agrees on **no classification for organic peroxide and, based on conclusive data, for self-heating substance and flammable solids**.

RAC agrees with the DS on no classification for explosive properties, as emamectin benzoate does not contain any chemical groups associated with explosive properties as given in section 2.1.4.3(a) of the CLP Regulation. Furthermore, data from test method A.14 are negative, which indicate that emamectin benzoate presents no danger of explosion when submitted to the effect

of a flame (thermal sensitivity), or to shock or friction (sensitivity to mechanical stimuli) according to this test method.

For the physical hazards 'pyrophoric solid', 'corrosive to metals' and as a substance which 'in contact with water emits flammable gases', RAC agrees with the DS on no classification **due to lack of data**.

RAC notes that emamectin benzoate does not contain any chemical groups associated with self-reactive properties as given in section 2.8.4.2(a) of the CLP Guidance, but measurement of heat of decomposition or self-accelerating decomposition temperature (SADT) is lacking. The substance has not been tested for thermal stability/self-reactivity (method A.2, OECD TG 103) although the self-ignition is measured at 395 °C. RAC notes that according to CLP, the classification of a self-reactive substance or mixture shall be performed in accordance with test series A to H as described in Part II of the UN RTDG, Manual of Tests and Criteria. The procedure for classification is described in Figure 2.8.1 of CLP. Therefore, these methods are only supportive. RAC agrees that it is unlikely that the substance will show self-reactive properties, given the absence of chemical groups associated with such properties. **In summary, RAC agrees with the DS on no classification for self-reactive properties**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify emamectin benzoate as Acute Tox. 3 for the three main routes of exposure. Emamectin was tested for acute toxicity using different salts as well as from different sources and purities. According to the DS and EFSA (2012), based on bioequivalence studies, the salts were generally found to be of similar acute toxicity and small differences in impurity profiles could not explain the variations in results.

The DS excluded from the assessment the study conducted with CF-1 mice, which was considered irrelevant for classification (B.6.2.1.1, Study 6). The CLH dossier summarised 9 acute oral toxicity studies (5 in rats; 3 in CD-1 mice), conducted according to OECD TG 425, TG 401 or similar, as well as 2 acute neurotoxicity studies in rats (no TG), all GLP compliant. The DS proposed to classify the substance as Acute Tox. 3; H301 with an ATE of 60 mg/kg bw, Based on the lowest (female) rat LD_{50} of 60 mg/kg bw (B.6.2.1.1, Study 4).

From 3 acute dermal toxicity studies in rats, conducted according to OECD TG 402 or no TG (B.6.2.1.1, Study 2) as well as one acute neurotoxicity study in rabbits (no TG) all GLP compliant, the DS selected the study with the lowest (male rats) LD_{50} range of 500-1 000 mg/kg bw (B.6.2.1.2, Study 3). The DS concluded on Acute Tox. 3; H311 and proposed to assign an ATE of 500 mg/kg bw for acute dermal toxicity.

From 3 acute inhalation toxicity studies in rats, conducted according to OECD TG 403 or equivalent and GLP compliant, the DS selected the study with the lowest (female) LC_{50} of 0.663 mg/L (B.6.2.1.3, Study 1). The DS concluded on Acute Tox. 3; H331 and proposed to assign an ATE of 0.663 mg/L for acute inhalation toxicity.

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

Oral toxicity

Effects in humans

Although not reported in the CLH report, there are two cases of human poisoning with emamectin benzoate formulations. Yen and Lin (2004) reported a non-fatal case of acute poisoning with 100 mL of formulated insecticide, consisting of 2.15 % w/w emamectin benzoate in 2,6-bis (1, 1-dimethylethyl)-4-methyl-phenol and 1-hexanol. The clinical manifestation was transient gastrointestinal upset with endoscopy-proven gastric erosion and superficial gastritis, mild CNS depression, and aspiration pneumonia. A second, more recent case report (Park, 2018), described a 75-year man who ingested intentionally 100 mL of a 2.15 % emamectin benzoate formulation. The patient experienced severe metabolic acidosis without CNS depression, ultimately leading to death. No further information is available to RAC.

Animal studies

A summary of acute oral toxicity studies is provided in the Table below.

Table: *summary of acute oral toxicity studies with emamectin salts*

Species	**Strain**	**Sex/n per dose**	**Emamectin salt tested**	**Vehicle**	**Purity (%)**	**Converted$ LD_{50} (mg/kg bw) [C.I.]**	**Ref.**
Rat	SD	3f	Emamectin benzoate technical	0.5 % w/w CMC solution in water	96.2	237 (f) [69-709]	B.6.2.1.1, Study 1
Rat (B.6.2.1.1, Study 6)	SD	5f and 5m	Emamectin hydrochloride	water	96.9*	100 (m) [73.5-140] 87 (F) [61.1-117]	B.6.2.1.1, Study 2
Rat	SD	5f and 5m	Emamectin benzoate hydrate	Aqueous MC	97.8	72 (m) 87 (f) 80 (m/f)	B.6.2.1.1, Study 3
Rat (bioequivalence study)	SD	5f	BMTE Emamectin benzoate hydrate salts	Aqueous MC	96.4 99.1	**60 (f) [51.3-71.8]** 66 (F) [53.6-79.8]	B.6.2.1.1, Study 4
Rat (exploratory study, combined with B.6.2.1.1, Study 9)	SD	5f	BMTE Emamectin benzoate salts	Aqueous MC	-	101 (f) [108.3-136.8] 103 (f)**	B.6.2.1.1, Study 5
Mouse	CF-1	f and m	Emamectin hydrochloride salt	water	96.9*	25 (m)	B.6.2.1.1, Study 6
Mouse (exploratory study)	CD-1	5 f	Emamectin benzoate hydrate salts	Aqueous MC	unknown	137 (f) [108-173] **124 (f)** [88-171]**	B.6.2.1.1, Study 7
Mouse	CD-1	f and m	Emamectin benzoate hydrate salt	Aqueous MC	97.6	153 (m) 178 (f) 165 (m/f)	B.6.2.1.1, Study 8
Mouse (exploratory study, combined with	CD-1	5f	BMTE	Aqueous MC	96.4 99.1	188 (f) 161 (f)	B.6.2.1.1, Study 9

B.6.2.1.1, Study 5)			Emamectin benzoate salts				
Rat (acute neurotoxicity study)	SD	10f and 10m	Emamectin hydrochloride salt	water	96.9	76 (m) [61.5-96] 80 (f) [63-118.6]	B.6.7.1, Study 1
Rat (acute neurotoxicity study)	SD	10f and 10m	Benzoate salt (unspecified)	water	94.2	> 29 (m/f)	B.6.7.1, Study 2

Note: $^{\$}$all LD_{50} values were converted to emamectin benzoate equivalents; BMTE: benzoate-methyl t-butyletherate solvate; CMC, carboxymethylcellulose; MC, methylcellulose; * 92.8 % B1a + 4.1 % B1b + 0.76 % propyl gallate added as antioxidant. **, the DS communicated to ECHA that in the CLH report a mistake of conversion factor was introduced for both 038W and 052S (1.14 instead of 1.16).

After oral administration, CF-1 mice are significantly more sensitive than CD-1 mice while SD rats are more sensitive than CD-1 mice. RAC also notes that deaths occurred as early as 30 minutes after exposure in CF-1 mice, which was also associated with bradypnoea and loss of righting reflex. Ataxia and whole body tremors occurred in all animals of all dose groups within 2 hours after exposure and persisted several days. In line with RAC (2010) and EFSA (2012), the results with CF-1 mice are not considered for classification. RAC nevertheless notes the there is an uncertainty related to the qualitative and/or quantitative sensitivity of different strains of rats or mice, including CF-1 mice.

According to the study results, the different salts of emamectin do not show significant differences in toxicity. In a comparative (bioequivalence) study (B.6.2.1.1, Study 4) selected by the DS as the basis of deriving the oral LD_{50} for classification, benzoate-methyl t-butyletherate solvate (MBTE) and benzoate monohydrate salts were tested separately in both female rats and female mice, and no major differences in toxicity were observed between the two salts.

RAC agrees with the DS that the LD_{50} values obtained in two species were systematically below 300 mg/kg bw and generally above 50 mg/kg bw for both males and females which, according to the CLP regulation, is the range for classification as Acute Tox. 3; H301. The purity of the batches or the vehicle used do not seem to influence the toxicity. In studies where both males and females were tested, there was little difference in sensitivity between the sexes.

In the most recent study (B.6.2.1.1, Study 1), performed according to GLP and OECD TG 425 (up and down procedure), the LD_{50} in female rats was 237 mg/kg bw with a rather wide 95 % confidence interval [C.I.: 69.5-755 mg/kg bw. The lower number of animals (1-3 females/dose) compared to other available studies (e.g. 10/sex in the acute oral neurotoxicity study, B.6.7.1, Study 1) could explain the wide confidence interval. In addition, dermal and inhalation studies conducted with emamectin benzoate showed that SD rats are less sensitive than Wistar rats, not tested by the oral route.

Based on the overall dataset, RAC is of the opinion that the LD_{50} for emamectin benzoate is likely closer to the lower limit of the Acute Tox. 3; H301 category than the upper limit (50 < ATE ≤ 300 mg/kg bw). The lowest converted LD_{50} is 60 mg/kg bw [C.I.: 51.3-71.8] (B.6.2.1.1, Study 4) in female rats.

Overall, RAC agrees with the proposal from the DS to **classify emamectin benzoate as Acute Tox. 3; H301, with an ATE of 60 mg/kg bw**.

Dermal route

There were three well-conducted acute dermal toxicity studies and one acute neurotoxicity study via the dermal route in the dossier (see Table below).

Table: *summary of acute dermal toxicity studies with emamectin benzoate salts*

Species	Strain	Sex/n per dose	Emamectin salt tested	Vehicle	Purity (%)	Converted[$] LD_{50} (mg/kg bw) [C.I.]	Ref.
Rat	SD	5f and 5m	Emamectin benzoate, technical	dry paste (75 % w/w mixture in distilled water)	96.2	> 1 754 (m/f)	B.6.2.1.2, Study 1
Rat	SD	5f and 5m	Emamectin benzoate hydrate salt, technical	water	96.4	> 2 000 (m/f)	B.6.2.1.2, Study 2
Rat	Wistar	5f and 5m	Emamectin benzoate, technical	undiluted	96.6	**500-1 000 (m)** **1 893 (f)**	B.6.2.1.2, Study 3
Rabbit, neurotoxicity	NZW	5f	Emamectin benzoate, technical (unclear)	saline	94.2	> 2 000	B.6.7.1, Study 3

In the study B.6.2.1.2, Study 3, using Wistar rats, conducted according to GLP and TG 402, RAC notes that the substance was applied undiluted (instead of as a dry paste moistened in water or in saline). It caused deaths in male rats at 500 mg/kg bw (1/5), 1 000 mg/kg bw (3/5) and 2 000 mg/kg bw (1/5). Deaths in female rats were only seen at 2 000 mg/kg (3/5). According to the study report, severe clinical signs (vocalization, irritability, tremors, tonic convulsion, piloerection, decreased activity, hunched back, discharge coloured, nose, area around eyes and incoordination) and weight loss were noted at all dose levels in males. Additionally, prone position, dyspnoea and lying on the side were noted in some animals dosed at 1 000 mg/kg and 2 000 mg/kg. There were no macroscopic findings at necropsy and no sign of skin irritation. Similar clinical signs were also observed in studies with SD rats (B.6.2.1.2, Studies 1 and 2).

In the absence of information on toxicokinetics and metabolism of emamectine benzoate between different strains and gender of rats via the dermal route, it is not possible to explain the higher sensitivity of male Wistar rats to emamectin benzoate. The low dermal absorption of emamectin benzoate in Rhesus monkeys (1.6 % of the applied dose) (Wrzesinski *et al*., 1997) and the high molecular weight (886 g/mol) of the substance do not favour systemic absorption via the skin. Nevertheless, there is no doubt that emamectin benzoate is more acutely toxic to Wistar rats relative to other rat strains via the dermal route.

No human data are available.

In conclusion, with the lowest LD_{50} ranging between 500-1 000 mg/kg bw, RAC agrees with the proposal from the DS to **classify emamectin benzoate as Acute Tox. 3; H311** for which the LD_{50} range is between 200 < LD_{50} ≤ 1 000 mg/kg bw. RAC notes that the available data may fit better with the ATE proposed by the DS in the CLH report (500 mg/kg bw). However, RAC considers that the **default ATE (300 mg/kg bw)** should be used in this case considering the lack of specific data on dermal LD_{50}.

Inhalation route

There were three well-conducted acute inhalation toxicity studies (nose only) in the dossier (see Table below).

***Table**: summary of acute inhalation toxicity studies with emamectin benzoate salts*

Species	Strain	Sex/n per dose	Emamectin salt tested	Vehicle	Purity (%)	Converted[$] LD_{50} (mg/kg bw) [C.I.]	Ref.
Rat	Wistar	5f or 5m	Emamectin benzoate, technical	None	96.2	m: between 1.049 and 1.981 mg/L f: 0.663 mg/L	B.6.2.1.3, Study 1
Rat	SD	5f and 5m	Emamectin benzoate, technical	None	96.4	Between 2.12 and 4.44 mg/L (m/f)	B.6.2.1.3, Study 2
Rat	SD	5f and 5m	Emamectin benzoate, technical	None	96.6	Not determined	B.6.2.1.3, Study 3

RAC agrees with the DS that the most reliable study (B.6.2.1.3, Study 1) is based on OECD 403 and GLP ('up and down' procedure). The first exposure concentration tested was 2.0 mg/L and was based on previous studies.

In male Wistar rats, the LC_{50} values were significantly higher than in females (between 1.049 and 1.981 mg/L and 0.663 mg/L, respectively). This difference between sexes cannot be explained. RAC notes a moderate difference in clinical signs at the different concentrations between males and females. During exposure, all animals of all exposure groups showed salivation and wet fur, associated with restraint and test substance staining around the snout. Reduced response to sound was observed in animals of the 0.506 mg/L dose group and above. After exposure, in addition to the symptoms mentioned above, decreased activity, hunched posture, piloerection, reduced response to sound, reduced righting reflex, and shaking was observed with nearly all animals of the high dose group, some males of the 1.049 mg/L dose group, and several females of the 0.506 mg/L dose group. Male animals recovered within 9 days and females within 11 days.

As observed via the dermal route, Wistar rats appear to be more sensitive to emamectin benzoate than SD rats. These studies did not precisely determine the LC_{50} but a NOAEL for neurotoxicity was established at 0.1 mg/L.

No human data are available.

In conclusion, RAC agrees with the proposal from the DS to select the lowest LC_{50} of 0.663 mg/L obtained with female rats (B.6.2.1.3, Study 1) as the basis for classification. This value is within the range of $0.5 < ATE \leq 1.0$ mg/L (dusts and mists) for Acute Tox. 3; H331. Overall, RAC considers that **classification as Acute Tox. 3; H331, with an ATE value of 0.66 mg/L** is warranted for emamectin benzoate.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS concluded that evidence of neurotoxicity fulfilled the criteria for classification as STOT SE 1 (nervous system). However, they did not propose a classification, considering instead that these effects were already covered by the classification as acute toxicity via all three routes of

exposure. The DS did not propose a classification for STOT SE 3 for transient target organ effects related to respiratory tract irritation or narcotic effects (drowsiness/dizziness).

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

The Tables in the background document (BD) under "Supplemental information – In depth analyses by RAC" provide an overview of specific, non-lethal target organ toxicity arising from a single exposure to emamectin benzoate by the three routes of exposure. Neurotoxicity is clearly the primary effect along with marked body weight loss. It can be observed that clear neurotoxicity occurs at lower exposure levels than those resulting in mortality, therefore it should be considered on the assessment of STOT SE. No information from humans was available to address this hazard class.

Oral route

Regarding STOT SE 1/2, RAC agrees with the DS that the available oral experimental studies show effects in the dose range relevant for classification for STOT SE 1 (< 300 mg/kg bw). In the three studies where body weights were recorded, marked body weight losses occurred after treatment and/or prior to death, but values seemed to return to normal after 7 and/or 14 days post-exposure in surviving animals. Histopathology was not performed in the any organs in the absence of gross necropsy observations.

Rats also consistently presented a dose-related but generally transient increase of signs of neurotoxicity (tremors, ataxia, bradypnoea, ptosis, decreased activity and lateral recumbency) from 26 mg/kg bw. Lesions in the brain, spinal cord and nerves were observed at ≥ 25 mg/kg bw, i.e. below doses where mortality occurred (ATE = 60 mg/kg bw) and below the guidance value for STOT SE 1 (C ≤ 300 mg/kg bw).

Dermal route

There were three well-conducted acute dermal toxicity studies in rats and one acute neurotoxicity study in rabbits in the dossier. None of the studies in rats reported histopathological changes in any organ. The well-conducted acute neurotoxicity study in rabbits reported histopathological degenerative lesions of the brain, spinal cord and peripheral (sciatic) nerve attributed to treatment (at ≥ 500 mg/kg bw). Changes included slight to moderate white matter degeneration in the optic chiasm, pons and/or cerebellar peduncles as well as degeneration in the spinal cord and the peripheral nerve. Nerve cell bodies were also affected in the pons and the spinal cord.

RAC further notes that:

- The marked to severe body weight loss observed in the acute dermal toxicity studies in rats and rabbits are at or above doses where mortality occurred (ATE = 300 mg/kg bw in rats and LD_{50} > 2 000 mg/kg bw in rabbits).
- Transient but dose-related increase in clinical signs of neurotoxicity (in particular tremors) and lesions in brain, spinal cord and nerves are observed in rabbits below doses where mortality occurred and below the guidance value for STOT SE 1 (C ≤ 1 000 mg/kg bw)
- Although clinical signs seem to reverse in some surviving animals (see "Supplemental information – In depth analyses by RAC"), neuronal degeneration confirmed by histopathology is considered irreversible and therefore a severe effect.
- Effects are specific to the substance due to its MoA.

Inhalation route

There were three well-conducted acute inhalation toxicity studies in rats. One study reported meaningful histopathological lesions to the nervous system at levels commensurate with those causing mortality.

RAC further notes that:

- The moderate and transient body weight loss observed in the acute inhalation toxicity studies in rats are generally at doses where mortality occurred (ATE = 0.66 mg/L).
- Transient but dose-related increase in generic clinical signs of neurotoxicity (tremor, ataxia, decreased activity) occurred in rats from the lowest concentration tested of 0.24 mg/L (B.6.2.1.3, Study 2) but not in another study (B.6.2.1.3, Study 3) at lower concentrations. They were accompanied by neuronal vacuolar degeneration in the brain and spinal cord at the higher doses with lethality (≥ 2.12 mg/L). Sciatic nerve degeneration was observed at ≥ 0.24 mg/L but with no clear dose response in this case. In B.6.2.1.3, Study 3, a NOAEL for neuronal degeneration in brain and nerve degeneration in sciatic nerve and/or spinal cord was established at 0.1 mg/L where they investigated doses of 0.01, 0.05 and 0.1 mg/L with no effects noted in any of these tested doses. In B.6.2.1.3, Study 1, no special attention has been given to findings in brain, spinal cord and/or sciatic nerve.
- Observations indicative of respiratory tract irritation are absent from the available studies.

The overall conclusion from the inhalation studies is that clear clinical signs of neurotoxicity were observed without pathological changes in the brain and spinal cord which occurred at higher concentrations often associated with dose levels causing lethality. The table below presents a summary of effects observed after a single administration of emamectin benzoate by different routes.

***Table**: Summary of effects observed after a single administration of Emamectin benzoate via different routes*

Route of exposure	Guidance value (GV) for STOT SE 1	Lesions to the nervous system < GV	Clinical signs of neurotoxicity
Oral	**≤ 300 mg/kg bw**		
Mouse		-	Yes
Rat		Yes (≥ 25 mg/kg bw)	Yes
Dermal	**≤ 1 000 mg/kg bw**		
Rat		No	Yes
Rabbit		Yes (≥ 500 mg/kg bw)	Yes
Inhalation	**≤ 1.0 mg/L**		
Rat		Yes (≥ 0.24 mg/L)	Yes (but no clear dose response relationship)

- : not available

Conclusion on STOT SE

STOT SE 1 or 2

Overall, RAC agrees with the DS that neurotoxicity fulfils the CLP criteria for classification as STOT SE 1 (nervous system) in mice, rats and rabbits since there is evidence of consistent and identifiable toxic effects below the GVs. However, in contrast to the DS, **RAC considers that STOT SE 1; H370 (nervous system) is warranted** because:

1. Neurotoxicity was seen after exposure via three different routes (oral, inhalation, dermal).
2. Dose levels inducing (at minimum) clinical signs of neurotoxicity were below the guidance value limits for STOT SE 1.

3. The dose levels inducing neurotoxic lesions were also well below those inducing mortality.
4. Lesions to the nervous system were seen in two species (rat, rabbit), considered severe and non-reversible.

STOT SE 3

Category 3 covers 'transient effects' occurring after single exposure, specifically respiratory tract irritation (RTI) and narcotic effects. Classification in Category 3 is primarily based on human data, which was not available for emamectin benzoate with the exception of two case reports of acute poisoning with emamectin benzoate formulations (Yen and Lin, 2004; Park, 2018). These cases provide information for emamectin benzoate products but not for the pure active substance.

Observations indicative of respiratory tract irritation were absent from the available studies. Therefore, RAC concludes that no classification is warranted for RTI.

According to the CLP criteria, narcotic effects that are observed in animal studies and that may include lethargy, lack of coordination, loss of righting reflex and ataxia can justify classification of substances for narcotic effects in Category 3. Some effects observed after acute exposure to emamectin benzoate are related to CNS depression, e.g. reduced alertness/ataxia, loss of reflex, lack of coordination and tremors. However, RAC considers that these symptoms are secondary to acute neurotoxicity as well as general toxicological effects rather than specific narcotic effects.

Overall, RAC also concurs with the DS' assessment, and considers that **classification of emamectin benzoate as STOT SE 3 is not warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation based on two well-conducted (OECD TG 404) negative *in vivo* studies in the rabbit (B.6.2.2.1, Study 1; B.6.2.2.1, Study 2).

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

Emamectin benzoate did not elicit any signs of dermal irritation in the first *in vivo* dermal irritation study with 3M/3F rabbits (B.6.2.2.2, Study 1). In the second, more recent study (B.6.2.2.1, Study 2), using 1M/2F rabbits, slight, transient erythema was observed on the skin of the three rabbits after application of 670 mg of the substance (dry paste, 75 % w/w mixture in distilled water). The scores for erythema rapidly declined over time (no erythema at 72 h post-application) and no signs of inflammation were observed.

Both studies used the same amount of emamectin benzoate. As the criteria for classification are not met, RAC agrees with the DS that **no classification for skin irritation/corrosion** is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed to classify emamectin benzoate as Eye Dam. 1; H318 based on two primary eye irritation studies performed according to OECD TG 405 in rabbits. In the most recent study (B.6.2.2.2, Study 1), the DS concluded that the effects met the criteria as Eye Irrit. 2; H319. However, in the second study (B.6.2.2.2, Study 2), severe signs of eye damage associated with other clinical signs (that lead to the premature euthanasia of 3 animals) occurred. Although these effects also fulfil the CLP criteria as Eye Irrit. 2, the DS considered that their severity (euthanasia of 3 animals) combined with the irreversibility of conjunctival discharge (within the 14-day period of observation) was sufficient to propose classification of emamectin benzoate as Eye Dam. 1; H318.

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the most recent *in vivo* eye irritation study (B.6.2.2.2, Study 1), at least two of three tested animals showed a response for iritis and conjunctival redness with a mean 24-72 h score ≥ 1 and a mean 24-72 h score ≥ 2, respectively. All reactions had reversed by day 7 (Table below).

***Table**: Mean scores following grading at 24, 48 and 72 h (B.6.2.2.2, Study 1)*

	1 h m/f/m	**Mean 24-72 h scores (m/f/m)**	**Day 4 m/f/m**	**Day 7 m/f/m**
Corneal opacity	0/0/0	0/0.33/1	0/0/1	0/0/0
Iritis	1/1/1	0.33/**1/1**	0/1/1	0/0/0
Conj. redness	2/2/2	1/**2/2**	0/2/2	0/0/0
Conj. chemosis	1/2/1	0.33/1.3/1.3	0/1/1	0/0/0

In the older study (B.6.2.2.2, Study 2), the responses of some of the rabbits (3m/3f) to emamectin benzoate (28 mg in the conjunctival sac) were so severe that 3 rabbits (2m/1f) were euthanized following the 72 h reading. In addition, ocular assessment was impossible for some endpoints due to severe chemosis. The three remaining rabbits survived until the end of the study (day 14). The Table below presents a summary of the mean scores following grading at 24, 48 and 72 h.

***Table*:** *Mean scores following grading at 24, 48 and 72 h (B.6.2.2.2, study 2)*

	1 h (m,m,m/f,f,f)	**Mean 24-72 h scores (m,m,m/f,f,f)**	**Day 6 (m,m,m/f,f,f)**	**Day 14**
Corneal opacity	0, 0, 0 / 0, 0, 0	0, -, - / -, 0, 0.33	0, E, E / E, 0, 0	0, E, E / E, 0, 0
Iritis	0, 1, 1 / 0, 1, 1	1, -, - / -, 1, 0.33	1, E, E / E, 1, 0	0, E, E / E, 0, 0
Conj. redness	2, 2, 2 / 2, 2, 2	3, 3, 3 / -, 3, 1.33	2, E, E / E, 2, 0	0, E, E / E, 0, 0
Conj. chemosis	2, 3, 2 / 3, 2, 3	2.6, 4, 4 / 3.6, 2.3, 0.33	1, E, E / E, 1, 0	0, E, E / E, 0, 0
Conj. discharge	3, 3, 3 / 3, 3, 3	3, 3, 2.3 / 3, 2.3, 0	2, E, E / E, 1, 0	1, E, E / E, 0, 0

Note: -, indicates that the sign could not be read at that time point due to chemosis; Conj., conjunctival; E, euthanasia.

The mean scores for conjunctival redness and chemosis over the period 24-72 h were ≥ 2.0 in at least 4/6 rabbits. The mean scores for iritis were ≥ 1 in 2/3 rabbits whereas for corneal opacity were below 1 for all animals. One female and one male still showed iritis up to day 6, conjunctival chemosis up to day 8 and conjunctival redness and discharge up to day 10, and the surviving male rabbit showed conjunctival redness up to day 10 and discharge up to the end of the study

(day 14). RAC was unable to determine whether the conjunctival discharge was reversible over a period of 21 days.

Both of these studies are considered acceptable and do not show any clear deficiencies. The difference in results between B.6.2.2.2 Study 1 and B.6.2.2.2 Study 2 is not easily explained from the available information though the amount of substance applied into the eyes (60 mg vs. 28 mg, respectively) was different as was the preparation of the test item before instillation (powder instilled as received vs. powder instilled after grounding to a finer powder). In addition, the lack of clinical signs in the recent study is probably related to the instillation of an ocular anaesthetic (tetracaine hydrochloride ophthalmic solution 0.5 %). RAC does not consider there is sufficient information to clarify why there are very different results in the two eye irritation tests

RAC notes that the CLP criteria cover substances that have the potential to seriously damage the eyes include those capable of causing severe reactions observed at any time during the test, as well as adhesion, pannus, and interference with the function of the iris or other effects that impair sight. In the older study (B.6.2.2.2, Study 2), emamectin benzoate produced effects that could interfere with the normal function of the eye. Congestion of the iris as well as corneal anaesthesia were observed up to 72 hours and one rabbit presented an unusual red spot (unknown origin) on days 8, 9 and 10 of the study. Some effects may not be fully reversible and the premature sacrifice of three rabbits with the most severe eye reactions raised particular concern. Furthermore, for those substances where there is a pronounced variability among animal responses, care should be taken in determining the classification (Section 3.3.2.7.2 of CLP).

In summary, RAC agrees with the DS that emamectin benzoate warrants classification as **Eye Dam. 1; H318 - causes serious eye damage**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There were no specific data available relating to the respiratory sensitisation potential of emamectin benzoate. The DS proposed no classification because of lack of data.

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the absence of information on respiratory sensitisation, RAC agrees with the DS's proposal of **no classification due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of emamectin benzoate was investigated in a local lymph node assay (LLNA) (B.6.2.2.3, Study 2) in the mouse and a guinea pig maximisation test (GPMT)

(B.6.2.2.3, Study 1). Both studies were negative. The DS proposed no classification based on conclusive data.

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

The LLNA and GPMT are summarised in the following table.

***Table**: Summary of the LLNA and GPMT results*

<table>
<tr><th colspan="3">Skin sensitisation studies</th></tr>
<tr><th>Type of study;
Reference</th><th>Method</th><th>Observations</th></tr>
<tr><td>LLNA
B.6.2.2.3, Study 2</td><td>OECD TG 429
GLP
0, 0.5, 1 and 2.5 w/v emamectin benzoate technical (96.2 %)
Vehicle: DMF
Strain: Mouse CBA/Ca/Ola/Hsd
4 females/group
Positive control: 25 % α-hexylcinnamaldehyde</td><td>Negative
Stimulation indices:
<table>
<tr><th>Treatment</th><th>SI</th></tr>
<tr><td>0.5 % Emamectin benzoate</td><td>1.3</td></tr>
<tr><td>1 % Emamectin benzoate</td><td>1.1</td></tr>
<tr><td>2.5 % Emamectin benzoate</td><td>2.1</td></tr>
<tr><td>Positive control</td><td>7.2</td></tr>
</table></td></tr>
<tr><td>GPMT
B.6.2.2.3, Study 1</td><td>OECD TG 406
GLP
Emamectin benzoate (> 95 %)
Intradermal induction: 5 % in 0.1 mL in Freund’s complete adjuvant/distilled water (1/1)
Topical induction: 7.5 % in petrolatum; dermal irritation induced by sodium lauryl sulphate (SLS) pre-treatment
Challenge: 1.25 % in petrolatum
Re-challenge: 0.5 % in petrolatum
Guinea pig (Hartley albino)
11 females/test group
10 females/control group
No concurrent positive control; the laboratory reliability check (1-chloro-2,4-dinitrobenzene) from the respective period was stated to have shown an acceptable response</td><td>Negative

Challenge (day 22): skin reactions in 3 controls and 2 treated groups (48 h)
Re-challenge (day 29): skin reactions in 1 controls and 1 treated groups (48 h)</td></tr>
</table>

In the LLNA performed according to OECD TG 429, animals were exposed to 25 µL of a 0.5, 1 or 2.5% w/v preparation of the test substance applied to the dorsal surface of each ear. The procedure was repeated daily for 3 consecutive days. The highest dose was selected based on the results of "*sighting studies run prior to the study in which single animals were exposed to 3 repeat topical exposures of 0.1, 0.5, 1, 2.5 and 5 % w/v emamectin benzoate. Only the 5 % w/v dose group showed signs of systemic toxicity*". RAC notes that no further information is provided in the study report regarding clinical signs or systemic toxicity observed after dermal contact.

The body weight gains were unaffected. Under the conditions of the LLNA test, emamectin benzoate is not considered a skin sensitiser.

In the GPMT performed according to OECD TG 406, no justification was provided for the choice of the highest concentration, the test is considered negative at 5 % (intradermal) and 7.5 % (dermal) induction concentrations.

As both studies were negative and neither of them showed any major methodological deficiency (except the relatively low induction concentrations used), RAC agrees with the DS and concludes that **no classification on skin sensitisation** is justified.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS summarised 17 repeated dose toxicity studies in different species (rat, dog, rabbit and mice) and of different durations, including developmental (pre- and post-natal) and reproductive toxicity studies in rats. The DS proposed to classify emamectin benzoate as STOT RE 1 (nervous system) based on neurotoxicity occurring in different parts of the central and peripheral nervous systems (axonal degeneration in the brain, spinal cord and various peripheral nerves) in rats and dogs. Clinical signs (e.g. tremors and abnormal movements like ataxia or incoordination) with an early onset always accompanied the histopathological features during the conduct of the studies.

According to the DS, the various studies suggest that the effects on the nervous system should be considered chronic rather than acute. Although the DS recognised similar hazards after single doses in rats and rabbits, the DS argued that acute effects were observed following treatment with relatively high doses (i.e. 10 mg/kg bw and up) while the chronic effects occurred at lower dose levels (i.e. 0.5 mg/kg bw/day and up). The DS concluded that neurotoxic effects observed after repeated exposure are more relevant for classification as STOT RE 1 (nervous system) than STOT SE 1.

The DS also reported a range of other effects occurring at higher dose levels than those resulting in neurotoxicity including perturbations of body weight, triglycerides and/or glucose in blood. However, the DS considered that these findings were not consistent between males and females, nor between studies, but may indicate slight perturbations of lipid and energy metabolism. Based on the emamectin benzoate MoA (GABA agonist), the DS expected an interference with energy metabolism, but considered these effects less critical than neurotoxicity and not fulfilling the classification criteria for STOT RE 1.

The DS did not specify a route of exposure or a specific concentration limit (SCL) for STOT RE 1 (nervous system).

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

Effects relevant for classification are summarised and compared with equivalent Guidance Values (GVs) in the Table below. Some studies have been omitted because they are supportive of the effects observed in the main studies (e.g. dose-range finding studies), but they are further

detailed in other sections of this opinion or are presented in detail by the DS in the Annex 1 to the CLH report. RAC notes that some dose levels were changed during the course of the studies and conversions from emamectin (base) compound to emamectin benzoate are not always reported in the CLH report as in the Annex 1 to the CLH report.

***Table**: Summary of effects and classification in relevant repeated dose toxicity studies*

Study reference	**Dose level expressed as emamectin benzoate (mg/kg bw/day)**	**Length of exposure**	**Equivalent guidance values**	**Classification**
B.6.3.3, Study 1	Dose level: 12.5/8.5 mg/kg bw/day (m/f) Critical effects: Brain lesions (m/f) Skeletal muscle, atrophy (m/f) Decreased bw gain and food consumption (m/f) Clinical neurological signs (fine tremor and splaying/limited use hindlimbs) (m/f)	90 days, oral CD rat	≤ 10 mg/kg bw/day (cat. 1) ≤ 100 mg/kg bw/day (cat. 2)	Cat. 1
B.6.3.3, Study 3	Effective Dose: 2.85 (m), 5.7/2.85 (f) mg/kg bw/day Critical effects: Brain lesions (m/f) Clinical neurological signs - ↓ arousal (m) - ↓ grip strength (transient: week 0-24; 70 to 90 %, f) - ↑ tremors/unkempt appearance (f) Increased plasma levels of triglycerides (m)	1 year, oral CD rat	≤ 2.5 mg/kg bw/day (cat. 1) ≤ 25 mg/kg bw/day (cat. 2)	Cat. 2
B.6.3.3, Study 4	Effective Dose: 0.6 mg/kg bw/day (m/f) Critical effects: Brain lesions (m/f) Skeletal muscle atrophy (m/f) Clinical neurological signs (m/f) Top dose (1.14 mg/kg bw): Premature sacrifice (week 2 or 6; m: 1/4; f: 2/4) Severe body weight loss (anorexia) (m/f) Decreased food consumption Thymus atrophy (m: 1/4; f: 2/4) Bone marrow, decreased number erythropoietic tissue (m: 1/4; f: 2/4)	14 weeks, oral Beagle dog	≤ 10 mg/kg bw/day (cat. 1) ≤ 100 mg/kg bw/day (cat. 2)	Cat. 1
B.6.3.3, Study 5	Effective Dose: 0.6 mg/kg bw/day Critical effects: Brain lesions (m/f) Muscle fibres degeneration (f) Clinical neurological signs (f, 1/4) Top doses 0.86/1.1 mg/kg bw: Premature sacrifice (day 49; m, 4/4) Eye retina cellular degeneration (3/4 in m/f) and eye optic nerve axonal degeneration (m/f)	1 year, oral Beagle dog	≤ 2.5 mg/kg bw/day (cat. 1) ≤ 25 mg/kg bw/day (cat. 2)	Cat. 1
B.6.5.1, Study 1	Effective Dose: 1.14 mg/kg bw/day	2-year, oral	≤ 1.25 mg/kg bw/day (cat. 1)	Cat. 1

Study reference	Dose level expressed as emamectin benzoate (mg/kg bw/day)	Length of exposure	Equivalent guidance values	Classification
	Critical effects: Brain lesions (m/f) Increased plasma levels of triglycerides (f) Effects on bodyweight gain (m/f)	SD rat	≤ 12.5 mg/kg bw/day (cat. 2)	
B.6.5.1, Study 2	Effective Dose: 14.3/ 8.6/ 5.7 (m), 14.3/ 8.6 (f) mg/kg bw/day Critical effects: Degeneration of sciatic nerve Clinical neurological signs (m) Increased mortality (ss, m/f) Decreased bw (m/f) Macroscopy: dermatitis (m/f), Spleen enlargement (m/f) Microscopy: dermatitis (m/f), Spleen ext. heam. (m/f), bone marrow, myeloid hyperplasia (m/f)	1.5 year, oral CD-1 mouse	≤ 1.6 mg/kg bw/day (cat. 1) ≤ 16.0 mg/kg bw/day (cat. 2)	Cat. 2
B.6.6.1, Study 2	Effective Dose: Parental: 2.1 mg/kg bw/day Developmental: 2.1 mg/kg bw/day Fertility: 2.1 mg/kg bw/day Critical effects: Brain lesions (m/f) Sciatic nerve (m) Reduced bw gain (m) Increased bw gain and food consumption (pre-mating, f) Reduced food consumption during lactation (f)	Dietary 2-generation study of reproductive toxicity (30 days) rat	≤ 30 mg/kg bw/day (cat. 1) ≤ 300 mg/kg bw/day (cat. 2)	Cat. 1
B.6.6.2, Study 1	Effective Dose: Maternal: 4.56 mg/kg bw/day Developmental: 4.56 mg/kg bw/day Critical effects: Maternal: decreased bw gain (from day 6 of gestation) Clinical neurological signs (tremors)	Oral developmental study (19 days) rat	≤ 47 mg/kg bw/day (cat. 1) ≤ 470 mg/kg bw/day (cat. 2)	Cat. 1
B.6.6.2, Study2a	Effective Dose: Maternal: 6.8 mg/kg bw/day Developmental: > 9.1 mg/kg bw/day Critical effects: Maternal: decreased bw gain (days 14-19) and food consumption (day 16-22).	Range finding -Oral developmental toxicity study (18 days) rabbit	≤ 50 mg/kg bw/day (cat. 1) ≤ 500 mg/kg bw/day (cat. 2)	Cat. 1
B.6.6.2, Study 2	Effective Dose: Maternal: 6.8 mg/kg bw/day Developmental: > 6.8 mg/kg bw/day Critical effects: Maternal: decreased bw gain (days 6-28) Mydriasis (from day 11 and up) Decreased pupillary reaction (GD11-23)	Oral developmental toxicity study (18 days) rabbit	≤ 47 mg/kg bw/day (cat. 1) ≤ 470 mg/kg bw/day (cat. 2)	Cat. 1

ss: statistically significant; GD: gestation day

Nervous system toxicity and neurological clinical signs

RAC agrees with the DS that the primary target organ of emamectin benzoate after repeated exposure is the nervous system. Additional details are provided in the section "Supplemental information – In depth analyses by RAC". Histopathological lesions (neuronal degeneration in the brain and the spinal cord) were observed in most subchronic and chronic toxicity studies and the predominant clinical signs of neurotoxicity included tremors and abnormal movements, characterized as ataxia or incoordination. Clinical signs tend to appear before histopathological lesions and the dog is the most sensitive species.

Skeletal muscle atrophy/degeneration

In dogs and rats, skeletal muscle atrophy/degeneration was observed. The skeletal muscle changes are consistent with neurogenic atrophy. These effects were generally graded as very slight to slight in severity. However, in a specific 14-week neurotoxicity study in rats (B.6.7.2, Study 4), very slight to marked atrophy was seen in the skeletal muscle of 3/7 male rats only, at the top dose of 5.7 mg/kg bw/day. These changes were correlated with neurotoxicity and functional observational battery test results and are considered by RAC as secondary to neurotoxicity and clinical signs.

Optical nerve and retinal tissue

The DS did not differentiate lesions observed in the optic nerve from these occurring in other nerves (e.g. peripheral, sciatic). Degenerative changes in the optic nerve were reported in subchronic toxicity studies in dogs (B.6.3.3, Study 4) and CD rats (B.6.3.3, Study 1) as well as in the chronic toxicity study in dogs (B.6.3.3, Study 5) but not in mice. In the subchronic study in CD rats (B.6.3.3, Study 1), damage to the optic nerve was observed in one female rat at a dose of 2.85 mg/kg bw/day. In the 1-year chronic study in rats (B.6.3.3, Study 3), no damage to the optic nerve was noted at doses up to 2.85 mg/kg bw/day. In the 14-week study in dogs (B.6.3.3, Study 4), damage to the optic nerve (2/4 and 3/4 in males and females, respectively) was observed at the top dose (1.0 mg/kg bw/day of emamectin base compound for days 14/15 to 91/92). In the 52-week toxicity study in dogs (B.6.3.3, Study 5), very slight to slight damage to the optic nerve was observed at the top doses (0.75 and 1.0 mg/kg bw/day of emamectin base compound). Therefore, the dog is the most sensitive species and sensitivity increases with study duration. In addition, optic nerve damage was also associated with very slight eye retina (ganglionic cells) degeneration at 0.8 and 1.1 mg/kg bw/day (2/4 in males and 1/4 in females; 3/4 males and 3/4 females, respectively).

RAC notes that the optical nerve lesions and in particular eye retina (ganglion cells) degeneration may accelerate the occurrence of glaucoma. Although these effects were very slight to slight, they were observed in two species and three independent studies with a dose-response observed in dogs.

Effects related to body weight

Effects on body weight gains were observed in several studies, as well as effects on levels of triglycerides and/or glucose in blood in the 1-year study in Crl:CD rats (B.6.3.3, Study 3). In the 2-year study in rats (B.6.5.1, Study 1), females exposed to 1.14 and 2.85 mg/kg bw/day had increased average weight gains (10 to 28 % compared to controls). A similar but less pronounced effect was observed in males (15 to 20 % compared to controls). The high dose males started to lose weight over the end of the study (weeks 53 to 85). Food consumption was slightly increased in these groups. As observed in the 1-year study (B.6.3.3, Study 3, SD rat), serum triglycerides were significantly higher in females in the mid and high dose groups and generally correlated with body weights. There was also an increased incidence of chronic proliferative cystitis of unknown origin in the male rats only in the high dose group compared to controls (incidence: 7, 7, 7 and 17 % in the control, low, mid and high dose groups, respectively).

However, these findings were not consistent between males and females, or between studies, but may indicate slight perturbations to lipid and energy metabolism. Since emamectin benzoate is a GABA agonist, interference with energy metabolism can be expected.

In addition, maternal body weight gains were increased during gestation at 0.68 and 4.1/2.85 mg/kg bw/day (11 and 15 % above controls, respectively) in the oral developmental neurotoxicity study in rats (B.6.7.3, Study 1). Analogous increases in body weight gain have been observed previously upon treatment with ivermectin and are generally characteristic of avermectins. As the mechanism by which avermectins increase body weight gain is unknown, these effects should be considered potentially adverse and not disregarded.

Other effects

Several other effects were reported by the DS after repeated exposure to emamectin benzoate but they were not discussed and compared with the criteria.

There were indications of effects on the thymus in the 14 week toxicity study in dogs (B.6.3.3, Study 4). In the high dose group, thymus atrophy was noted in 1/4 male and 2/4 female dogs vs 0/4 in control. Thymus atrophy was accompanied by decreases in the number of erythropoietic cells in the bone marrow. Dogs in the high dose groups displayed severe signs of neurotoxicity, and the effects on the thymus could be secondary to neurotoxicity. No effects on the thymus were observed in the 1-year dog study (B.6.3.3, Study 5). Since these effects were observed in one study only, they were not considered further for classification.

A dose-related increase in the incidence (and severity) of bacterial infection producing skin lesions was seen in mice in the 1.5-year carcinogenicity study (B.6.5.1, Study 2). There was no indication of direct skin toxicity. Since these effects were not observed in other studies, they are not considered further for classification.

In both repeated dose toxicity studies in dogs (14 and 52 weeks), premature sacrifice of several males and females occurred at the top doses. In the 14 week study, two dogs (1M and 1F) dosed at the top dose were killed in extremis in week 2 and another female dog dosed at the reduced level of 1.0 mg/kg bw/day (base compound) was killed in week 6 (B.6.3.3, Study 4). Prior to death, the animals were observed with tremors, mydriasis, anorexia, lethargy and recumbency due to severe clinical neurological signs. In the 52 week study (B.6.3.3, Study 5), all animals showed signs of severe toxicity at the top dose, and were killed after 19 daily doses. In the mid-dose group, physical signs appeared from week 5 in males and consisted of fine whole body tremors, mydriasis and stiffness of hind legs. Because of these signs of overt toxicity, all males in this group were killed after 49 days.

Conclusion on classification

RAC recognises that the overall adverse health effects are severe, consistent and identifiable, affecting the function of the nervous system. From a toxicokinetic point of view, the available studies in rats show that emamectin has a low bioaccumulation potential, has a limited gastro-intestinal absorption and, compared to other organs, shows very low residue levels in the brain and the spinal cord. After oral administration, the majority of orally administered radioactivity was for example excreted in faeces (90 % or more over 168 h), with less than 3 % excreted in bile and only 0.1-0.3 % in urine. Emamectin is not metabolised to a substantial extent (B.6.1.1, Studies 1-3).

Morbidity or unscheduled death resulted from repeated exposure at or above 0.87 mg/kg bw/day in dogs warrants consideration for STOT RE. Mortality fits with the criteria of significant toxic effects of relevance to human health and this may be used to support classification under STOT RE. It is also noted by RAC that morbidity leading to premature sacrifice or unscheduled death occurred in dogs after repeated exposure at dose levels relevant for classification as STOT RE (e.g. after 19 days of 1.0 mg/kg bw emamectin base compound, B.6.3.3, Study 5). According to

CLP Regulation, Annex I, section 3.9.2.7.3, morbidity or death resulting from repeated or long-term exposure "can be taken into account for classification as STOT RE" but the Regulation does not specify that it can be added to the hazard statement in classification and labelling, because mortality is not recognised as a specific target organ.

RAC concurs with the conclusion of the DS that classification as STOT RE 1 is warranted. RAC recognises that the nervous system (both central and peripheral) is the primary target organ although other organs or systems are also of concern. Although histopathological changes in the nervous system were generally graded very slight to slight in severity, they occur at dose levels far below the suggested guidance values (sometimes by one order of magnitude), are clearly dose-related and are considered irreversible.

RAC agrees with the DS not to indicate the route of exposure. This is supported because in addition to the oral route, it may be assumed inhalation and dermal exposure will lead to serious effects, which are clearly indicated by the acute toxicity data for the three different routes. RAC also notes that the closely related substance abamectin showed severe toxicity after repeated exposure by inhalation well below the guidance values (RAC, 2010).

The DS did not propose a specific concentration limit (SCL) for neurotoxic effects. RAC also notes that abamectin has an entry in Annex VI to the CLP Regulation for STOT RE 1 (nervous system) with SCLs (STOT RE 1; H372: C ≥ 5 % STOT RE 2; H373: 0.5 % ≤ C < 5 %).

RAC is of the opinion that an SCL is also required for emamectin benzoate in view of the very steep dose response in dogs where histopathological changes in central and peripheral nervous system and premature sacrifice occurred at dose levels above 0.6 mg/kg bw/day. The NOAEL was only half this dose i.e. 0.29 mg/kg bw/day (expressed as emamectin benzoate) in both the 14 and 52-week dog studies.

From the dataset, RAC considers the dog as the most sensitive species for repeated dose toxicity. The relevant oral effective dose is 0.6 mg/kg bw/day expressed as emamectin benzoate. Therefore, SCLs may be calculated as follows:

<u>14-week dog study (B.6.3.3, Study 4):</u>

SCL for STOT RE 1 = ED/GV1 (0.6/10) × 100 = 6.0

SCL for STOT RE 2 = ED/GV2 (0.6/100) × 100 = 0.6

According to the CLP Guidance (v.5, July 2017), the resulting SCL should be rounded down to the nearest preferred value (1, 2, or 5). The SCLs proposed by RAC are therefore 0.5 % and 5 %.

Thus, in agreement with the DS, RAC concludes that emamectin benzoate fulfils the criteria for classification for effects on the nervous system and mortality. In conclusion, emamectin benzoate warrants classification as **STOT RE 1; H372 (nervous system)**. In addition, RAC proposes **SCLs** based on the repeated dose dog studies as follows: **STOT RE 1; H372: C ≥ 5.0 % w/w; STOT RE 2; H373: (0.5 % ≤ C < 5.0 % w/w).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The genotoxic potential of emamectin benzoate has been investigated both *in vitro* and *in vivo* assays. In *in vitro* studies, an Ames test (B.6.4.1, Study 1), two *in vitro* chromosome aberration

assays (V79, B.6.4.1, Study 2; and CHO cells, B.6.4.1, Study 3) and an *in vitro* alkaline elution/rat hepatocyte assay (B.6.4.1, Study 4) were negative.

The *in vivo* dataset, consisting of a chromosome aberration assay (B.6.4.2, Study 1) in male mice, was negative.

According to the DS, all *in vitro* and *in vivo* studies were reliable and were clearly negative. Therefore, the DS proposed no classification based on conclusive data.

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

The CLH dossier included five studies on mutagenicity, four of which were performed *in vitro* and one *in vivo*. All of the *in vitro* studies were conducted using protocols that resembled current OECD TG. Concerning the *in vivo* chromosomal aberration study in male mice, the study design also resembled OECD TG 475. RAC notes that the study selected the most sensitive sex (males) and used a highest oral dose (80 mg/kg bw) close to the maximum tolerated dose in mice. Although no toxicokinetics data are available, there was a dose-related depression of the mean % mitotic index up to 24 hours after oral administration, suggesting that exposure of the bone marrow had occurred.

RAC agrees with the DS that **emamectin benzoate does not fulfil the criteria for classification for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of emamectin benzoate was investigated in two carcinogenicity studies, one in Crl:CD rats and one in Crl:CD-1 mice. According to the DS, the long-term oral toxicity/carcinogenicity study with rats (0, 0.29, 1.14 and 5.7/2.85 mg/kg bw/day during 104 weeks) and the oral carcinogenicity study with mice (0, 0.57, 2.85 and 14.3/8.6/5.0 (m), 14.3/8.6 (f) mg/kg bw/day during 79 weeks) were negative (B.6.5.1, Study 1 and B.6.5.1, Study 2). Both species were administered emamectin benzoate hydrate via the diet.

Overall, the DS concluded that there was no evidence of carcinogenicity in either the rat or the mouse at any of the dose levels used. In addition, no increases in pre-neoplastic changes were observed. No classification was proposed by the DS

Comments received during the consultation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

In the rat carcinogenicity study (B.6.5.1, Study 1), no tumours were observed. The only treatment-related non-neoplastic finding was an increased incidence of chronic proliferative cystitis (of unknown origin) in the male rats only in the high dose group compared to controls

(incidence: 7, 7, 7 and 17 % in the control, low, mid and high dose groups, respectively). This change was characterised by the presence of a very slight to marked degree of transitional epithelial thickening or hyperplasia with chronic inflammatory cell infiltrate in the adjacent sub-epithelial connective tissue and was sometimes accompanied with urolithiasis. There was no neoplasia in the study.

In the mouse carcinogenicity study (B.6.5.1, Study 2), dose levels were reduced due to the severity of clinical signs and mortality occurring in males. For males, it was reduced from 14.3 to 8.6 mg/kg bw/day beginning of week 9 and then to 5 mg/kg bw/day during week 31. For females, the high dose level was reduced from 14.3 to 8.6 mg/kg bw/day during week 48. At the top dose level, there was an increased incidence of mortality (males and females), marked decreased weight gain, clinical signs of neurotoxicity (tremors), increased incidence of skin lesions (secondary to bacterial infections), changes in haematological parameters and increased relative organ weights observed in high dose mice. However, there were no treatment-related increases in tumour incidence in mice.

As no treatment-related increase in tumour responses were reported and no concern was identified for cell mutagenicity, RAC supports the DS's proposal that **emamectin benzoate does not fulfil the criteria for classification for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for sexual function and fertility based on the results obtained in a dietary 2-generation study in Crl:CD rats (B.6.6.1, Study 2) which was also preceded by a range finding study (B.6.6.1, Study 1). According to the DS, the only reproductive toxicity effects related to reduced fecundity found solely in the top dose group (4.1 mg/kg bw/day reduced to 2.1 mg/kg bw/day). Although a clear dose response relationship was absent during first and second mating giving rise to F1 animals, a steep dose response relationship (for maternal toxicity), was observed at the highest dose in animals producing the F2 generation. At this dose, mating behaviour was considered to be influenced by parental effects not directly related to reproduction but related to neurotoxicity, exacerbated during post-natal development. Therefore, the DS considered that the effects on mating behaviour do not warrant classification.

The DS also proposed no classification for developmental toxicity. The DS summarised several studies including two range finding and two prenatal developmental toxicity studies in rats and rabbits as well as a developmental neurotoxicity study in rats. According to the DS, there was no strong evidence of teratogenicity in these developmental toxicity studies. The DS considered most findings were associated with the presence of maternal toxicity (and thus not considered sufficient for classification) or secondary to neurotoxicity. For developmental (post-natal) neurotoxicity, they considered most of the effects in pups and neonatal rats (neurotoxicity, growth retardation, delays in sexual maturation, behavioural effects) were related to a unique high susceptibility period to neurotoxicity in young animals and not relevant for classification. Since this susceptible period (defined as that time following birth and early maturation) in rodents which displays limited P-glycoprotein expression is not present in humans, the effects observed were not considered relevant for human. The DS therefore did not consider any of these effects for classification.

The DS also concluded that the main 2-generation study did not report any adverse findings occurring via lactation and did not propose to classify emamectin benzoate for effects on or via lactation.

Comments received during the consultation

One MSCAs and 1 manufacturer commented on reproductive toxicity.

Sexual function and fertility

A manufacturer supported the DS proposal for no classification. They submitted a position paper in which they emphasised that the apparent effects on fertility in the dietary 2-generation study in rats (B.6.6.1, Study 2) at the top dose level only (4.1/2.1 mg/kg bw/day) could be explained by impaired mating due to neurotoxicity. Three lines of evidence formed the basis of this hypothesis:

- Treatment-related lower fertility indices and evidence of neurological effects (clinical signs and/or neuropathological lesions) were restricted to and concomitantly found in the top dose group only.
- The percentage of females exhibiting characteristics of pseudo-pregnancy (PSP) was consistently higher in the top dose group. According to the manufacturer, PSP can be induced by ineffective male copulation which is consistent with probable neurological impairment in males.
- Calculation of fertility indices excluding pseudo-pregnant females showed no treatment-related effect on fertility. This last point in particular supports the view by the manufacturer that animals capable of mating effectively, i.e. not sufficiently impaired by the neurological effects of emamectin benzoate, exhibited no adverse effects on reproductive performance.

The manufacturer also argued that the more marked effect on fertility indices in the F1 mating for the F2 generation is likely to be related to the sequelae of the increased sensitivity of the F1 animals to the neurotoxicity of emamectin during the neo-/post-natal period. As explained previously, the effects in the neonatal rat are considered to be caused by a direct, specific susceptibility to neurotoxicity, and not by a maternally mediated effect.

One MSCA requested clarifications on different aspects since they considered that data might suggest classification for sexual function and fertility as Repr. 2; H361f. The MSCA suggested, in contrast to the fertility index, a reduced fecundity index is more difficult to explain by a neurological component since copulation has occurred in the reference group, if proved by the presence of a plug/sperm positive females. Further, the MSCA mentioned a possible treatment-related effect on a small proportion of females involving an inhibition of some reproductive processes that normally occur after successful mating.

In their response, the DS emphasised that reduced fecundity/ fertility is observed in the top dose group only and reached statistical significance in the top dose F1-animals only, upon generating the F2-generation. The DS also referred to the possibility of some females with PSP. Therefore, the DS confirmed the view that the observed effects on fecundity/fertility are secondary to neurotoxicity and that classification is not warranted in this case.

Development

A manufacturer submitted during the consultation a second position paper arguing that the effects in the neonatal rat are considered to be direct toxicity resulting from direct exposure to emamectin via the milk and diet, and not to a maternally mediated effect. The higher susceptibility of neonatal rats is based on literature data (Lankas *et al*. 1989, Matsuoka *et al*. 1999, Betz and Goldstein, 1981) as reported previously (RAC, 2010; EFSA, 2007; EFSA, 2012). The manufacturer also provided a more recent reference (Lam *et al*., 2015) where the authors examined P-glycoprotein levels across the human foetal, neonatal and adult periods. They showed that brain P-glycoprotein amount to 35 % of adult levels at 20-26 weeks of gestation, to 43 % of adult levels by weeks 36-40 of gestation, and to 58 % of adult levels at 0-3 months of

age. Due to these quantitative differences, the manufacturer reiterated that the toxicity seen in the neonatal rat would not be expected to be seen in a neonatal human.

An MSCA provided comments on the developmental toxicity studies with a particular emphasis on a possible classification as Repr. 2; H361d based on malformations in the range finding study in rabbits (B.6.6.2, Study 2a) where one cleft palate and two hydrocephalus at the top dose (9.12 mg/kg bw/day, expressed as emamectin benzoate) were noted. The MSCA referred to RAC opinion on abamectin/avermectin B1a, classified as Repr. 2; H361d (RAC, 2010). For emamectin benzoate, skeletal malformations and variations in foetuses are also reported in the main study in rabbits (B.6.6.2, Study 2). The MSCA stressed that these malformations should not be automatically discounted in association with maternal toxicity and should be compared with appropriate historical control data (HCD).

The DS responded that these observed developmental effects do not warrant classification. With regard to the malformations and foetal anomalies, they should be considered incidental (since they are also within the laboratory HCD) and/or probably secondary to maternal toxicity. However, the DS recognised that no detailed HCD were available for assessment. The DS considered that the closely related substance abamectin was classified as Repr. 2; H361d based on different reasons i.e. there was a slightly higher incidence of clubbed forefoot malformations in rabbit foetuses in the top dose group (2 mg/kg bw/day) compared to the control (5 cases vs 1 case). There were no such findings with emamectin. The malformations seen in the range finding developmental rabbit study are in the presence of clear maternal toxicity. Finally, the DS referred to the WHO Joint Meeting on Pesticide Residues (JMPR, 2011) which also concluded that emamectin was not teratogenic in rats or rabbits.

Developmental neurotoxicity

According to the MSCA, some effects in pups should also be clarified in relation to maternal toxicity and the high susceptibility of neonatal rats due to limited P-glycoprotein expression until 20 days after birth should be discussed under developmental neurotoxicity. The DS agreed with the MSCA.

Effects on or via lactation

No comments were received on this hazard class.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The DS proposed no classification for fertility and sexual development based on the results obtained in a dietary 2-generation study in the rat (B.6.6.1, Study 2) which was also preceded by a range-finding study (B.6.6.1, Study 1). Summary of effects in both studies is presented in the background document.

Effects on fecundity/fertility indices

RAC agrees that the 'fecundity index' (number of pregnant females/number of females with confirmed matings × 100) is not mentioned in CLP nor in OECD TG 443. The term 'fertility index' is defined in the CLP Regulation as the number of animals with implants/number of matings × 100 and the mating index as the number of animals with seminal plugs or sperm/number mated × 100. The latter two indices can also be affected by toxicity occurring in males.

Generation of the F1A litter revealed lower fertility indices in all groups administered emamectin, which was largely due to fewer full-term pregnancies from those animals exhibiting signs of

mating. These changes did not achieve statistical significance ($p > 0.05$) at any dose level. There was a lack of a coherent dose-related response and the fertility index in all treated groups was within or close to the historical control range (mean 87 %; range 57-100 %). To investigate these findings further, the F0 parents were mated a second time to produce an F1B litter. This time, a lower fertility index was again observed at the highest dose level suggesting that reduced fertility at this top dose level may be related to test substance administration. There was a more marked reduction of the fecundity indices in generation of the F2 litters at the top dose level. These data are consistent with the higher incidence of neurological signs observed in the F1 parental generation.

RAC finds plausible that a lower fertility index may partly be male-mediated and these effects could be secondary consequence of neurotoxicity to the male leading to ineffective copulation. Neuropathological lesions were noted in F0 and F1 parents together with evidence of neurological effects at the top dose level in F1A, F1B and F2 pups – manifesting as tremors, hind-limb extension and limited use of hindlimbs. Physical impairment of the top dose male rats may thus have contributed to inadequate copulation. This is in line with the MSCA comment during the consultation that marked neurological disorders could induce a reduced pregnancy.

Presence of plug/sperm was confirmed in all cases. According to the original study report, the number of 'infertile' males was comparable across all groups (2-3-1-1) whereas there were approximately 20 % of mated females at the top dose, which failed to produce a pregnancy after successful mating (see Table below).

RAC concurs with study authors and the comment from the MSCA during consultation that the lower fertility indices may be male-mediated and a secondary consequence of the neurotoxic effects of emamectin. According to the applicant, maternal bodyweight profiles during gestation indicated that many of the affected females were likely to have been pseudo-pregnant rather than actually non-pregnant. The percentage of females exhibiting PSP characteristics was consistently higher in the top dose group and particularly marked in the F1a mating (35 %, 8/23 matings), which exhibited a concordance with the high incidence of neurological signs noted in the F1a generation (figure below, PSP).

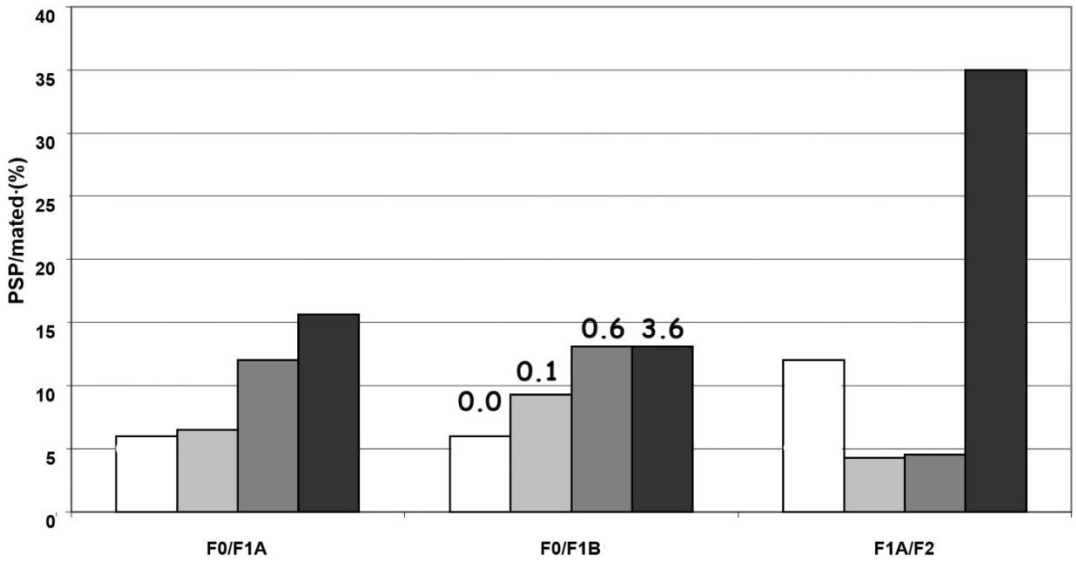

Figure: Percentage of female rats that showed evidence of pseudopregnancy

Re-calculation of the fecundity index by the applicant (in the absence of animals concluded to be pseudo-pregnant) presents no significant effect at any dose level in the generation of the F1a litter (see the Table below). The lowest fecundity index value was seen at the low dose level and

the highest value at the mid dose level, indicating a normal range of fecundity index values across the group and across the two generations.

Table: *(re-)calculated fertility indices for each of the three matings performed in the emamectin benzoate rat 2-generation study*

	0 mg/kg	**0.11 mg/kg**	**0.68 mg/kg**	**4.1/2.1 mg/kg**
F0/F1a				
Fertility index	91	67	76	67
Fecundity index	91	71	76	71
Fecundity index, recalculated	97	76	86	85
Mated animals considered to be pseudopregnant	2	2	4	5
F0/F1b				
Fertility index	85	85	81	70
Fecundity index	88	88	84	74
Fecundity index, recalculated	93	97	96	85
Mated animals considered to be pseudopregnant	2	3	4	4
F1a/F2				
Fertility index	80	80	84	48
Fecundity index	80	87	95	52
Fecundity index, recalculated	91	91	100	80
Mated animals considered to be pseudopregnant	3	1	1	8

Note: HCD for fertility index: mean 87 %; range 57-100 %

PSP can occur in receptive (pro-oestrous/oestrous) females after sterile or inadequate mating (Hafez, 1970). When the cervix and vagina are stimulated in receptive females, either mechanically or by coitus, prolactin is released from the anterior pituitary, which in turn, triggers the corpora lutea to secrete progesterone. Secretion continues for approximately 13 days and during this time, oestrous cycling ceases, new follicles do not mature, and the uterus undergoes endometrial growth. Up to this point, bodyweight gain in pseudopregnant and pregnant females is very similar. If fertilisation and implantation have occurred, the placenta now takes over progesterone production for the remainder of the pregnancy. If fertilisation has not occurred, oestrous cycles resume, the uterus reverts to the non-activated state and there is a characteristic loss of bodyweight thus accounting for the characteristic pattern of weight gain up to day 12, followed by a weight loss on subsequent days. Hyperprolactinemia is known to cause PSP in rodents, characterised by a persistent dioestrus lasting approximately 12 days, followed by a return to sexual receptivity. Some neuroleptic agents and dopamine receptor antagonist have been shown to modulate prolactin levels. 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 mating leading to PSP usually indicates inadequacy of the male. In the rat, following ejaculation, transport of sperm from the vagina to the uterus is not automatic. Various behavioural and physiological factors influence this transport process and these may be susceptible to neurological interference. In the emamectin multigeneration study high dose group, neuropathological lesions were noted in F0 and F1 parents together with evidence of neurological effects at this dose level in F1A pups – manifest as tremors, hind-limb extension and limited use of hind-limbs. Although not specifically assessed in this study, according to the applicant, it is likely that the high dose parental males had some hind-limb weakness given the above data and previous findings in the 13/14 and 53 week toxicity studies. 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.

Sexual maturation of F1 pups in the developmental neurotoxicity study in rats

In the oral developmental neurotoxicity study in female rats (B.6.7.3, Study 1), a delay of 3.6-3.7 days in preputial separation (PS) and vaginal opening (VO) was observed in pups of the high dose group of 2.85 mg/kg bw/day. No statistical tests were conducted and no historical control ranges were provided. There is no evidence to determine if the delays have been caused by direct effects on the genital tract or by effects on systemic endocrine function. In addition, associations with body weight or body weight gain is complicated by the fact that emamectin benzoate perturbs the body weight gain with or without changes in food consumption of females.

RAC notes:

- The delay in sexual maturation may be secondary to a clear reduction in the rate of body weight development rather than a direct effect of emamectin benzoate. Prior experience with other substances considered by RAC (e.g. fluxapyroxad) where there were very clear and more pronounced reductions in the rate of post-natal body weight development across two-generations showed little to no effect on pubertal milestones, i.e. no delay in time to preputial separation in males or vaginal opening in females. However, in this case there are substantial reductions in mean pup body weight (-40 % relative to controls), in both sexes by PND 21 that this factor must be taken into account. Time-to-puberty endpoints in the context of post-natal body weight changes always need to be assessed carefully.
- The rat 2-generation study does not inform further on these endpoints since pubertal data were not measured.

Effects on vaginal opening (VO)

The estimated mean day of occurrence of VO/litter in pups and mean litter body weights are presented in the Table below.

***Table**: Estimated mean day of occurrence of VO in function of pup body weights*

		Dose group (mg/kg bw/day)			
		0	**0.11**	**0.68**	**2.8**
Number of pups	N	82	86	81	87
Day of age for VO (estimated)*	Mean	33.7	33.4 (-0.3)	33.1 (-0.6)	**37.4 (+3.7)**
PND 37: % animals not having attained VO	Mean	0	1	0	**35.8**
Mean body weight of female pups at PND 21 pre-weaning (g)	Mean	64.9	62.5	63.5	**37.9 (-41.6%)**
Total weight change (1-7 weeks post-weaning) (g)		207	196	195	**170 (-18 %)**

* VO assessed on PND 31, 34 and 37; % animals positive on each date recorded and used to estimate the day of occurrence. Actual days of occurrence were not measured. Not possible to analyse via Kaplan-Meier curves.

VO was delayed at 2.85 mg/kg bw/day emamectin benzoate by 3.7 days (estimated), which is, according to RAC, substantial. RAC further notes that in the high dose group, 30 out of 87 pups (35.8 %) had not achieved the criterion of VO by day 37. It is unclear how the estimated mean day of occurrence of VO was calculated but it may have been underestimated. An analysis of the mean pup body weight showed that there was a substantial reduction in body weight relative to controls by PND 21 and this may have had a bearing on the delayed VO.

RAC notes that reproductive organ weights were unaffected by treatment and there were no abnormalities found at necropsy related to treatment.

Effects on preputial separation (PS)

The table below presents the estimated mean day of occurrence of preputial separation/litter in pups and mean litter body weights.

Table: E*stimated mean day of occurrence of preputial separation in function of pup body weights*

		Dose group (mg/kg bw/day)			
		0	**0.11**	**0.68**	**2.8**
	N	79	89	88	87
Day of age for PS (estimated)*	Mean	44.8	44.9 (+0.1)	44.8 (+0)	**48.4 (+3.6)**
PND 47: % animals not having attained PS	Mean	5.1	8.7	9.0	**46.0**
Body weight of male pups at PND 21 pre-weaning (g)	Mean	67.1	65.0	65.1	**40.3 (-40 %)**
Total weight change (1-7 w post-weaning) (g)	Mean	371	371	367	**309 (-17 %)**

* PS assessed on PND 39, 43 and 47; % animals positive on each date recorded and used to estimate the day of occurrence. Actual days of occurrence were not measured.

PS was delayed at 2.85 mg/kg bw/day by 3.6 days. RAC further notes that in the high dose group, 40 out of 87 pups (46 %) had not achieved the criterion of PS by day 47. An analysis of the mean pup body weight showed that there was also a substantial reduction in body weight relative to controls by PND 21 and this may have had a bearing on the delayed PS.

Conclusion and comparison with the CLP criteria

Effect on fertility

In the main 2-generation reproductive toxicity study (B.6.6.1, Study 2), RAC considers the low fertility index as sufficient to raise concern regarding the capability of treated females to become pregnant. The effects on fertility and gestation indices 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 ($p > 0.05$) at any dose level. Furthermore, given the lack of a coherent dose-related response and the fact that the recalculated gestation index in all treated groups was within the historical control range provided during consultation (mean 87 %; range 57-100 %), 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 PSP 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. 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. PSP was only assessed by individual body weight gains. RAC notes that increased body weight gains, which could be interpreted as pregnancy in this experimental setting, occurred in female rats on other studies with emamectin benzoate independently of mating.

RAC however also notes that in a developmental neurotoxicity study (B.6.7.3, Study 1) with rats given emamectin benzoate by gavage at doses of 0, 0.11, 0.68 or 4.1/2.85 mg/kg bw/day, 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.

Effect on sexual maturation

Under CLP, it is recognised that adverse effects on sexual function and fertility include effects on the onset of puberty. This criterion would appear to be satisfied for emamectin benzoate, since it significantly delays the time of onset for PS in males and VO in females.

Published literature generally shows that delays in pubertal endpoints by substances due to endocrine-mediated mechanisms occur together with numerous other effects. For example, known anti-androgens responsible for significant delays in PS in males include flutamide, prochloraz, and vinclozolin but the effects are not solely confined to one specific event but occur together with other evidence that may include changes in nipple retention, anogenital distance/anogenital index and sex organ weights, as well as gross and histopathological findings. In female rats, atrazine, propazine and esfenvalerate prolong or delay vaginal opening by a number of days, often through centrally acting mechanisms that perturb the hypothalamic-pituitary control responsible for puberty attainment. These other effects are not apparent or have not been measured in rats treated with emamectin benzoate. The fact that both male and female pubertal endpoints are delayed may indicate a more general central acting mechanism or general toxicity. There is no mechanistic data however to explain the delayed attainment of the pubertal endpoints and the data are not sufficiently robust to perform a more in-depth statistical analysis because the end-points have been estimated rather than actually measured on an individual animal basis.

Reductions in body weight during post-natal development are known to cause delays in the onset of puberty. For both the top dose F1 female and male pups there is evidence of a delay in growth amounting to a ~ 40 % reduction relative to concurrent controls by PND 21. Post-weaning, male and female pup weights were decreased in lactation week 7 (22 % for males and 25 % for females). A significant decrease in pup weight gain over lactation period week 1-7 was observed in males and females of the high dose group. Therefore, RAC considers that the delayed pubertal effects seen in both sexes may be explained based on body weight change alone, although it cannot be excluded that these effects are treatment related. Emamectin benzoate clearly affects the development or time to attainment of puberty but it is plausible that this may be secondary to a general delay in growth rate in this particular case.

RAC also considers that, since there is no evidence of toxicity to reproductive organs in various species, the effects on fertility do not warrant classification in category 1A or 1B. In addition, in females that gave birth, there was no change in total implantations, pre- and post-implantation losses or in total and live pups over two generations. No effect reproductive organs were observed in the studies in which this was measured; nor any effect on follicle counts in the ovaries.

Overall, RAC considers the lower reproductive indices at the top dose as an adverse effect, however, RAC agrees with the DS that the percentage of females exhibiting characteristics of PSP was consistently higher in the top dose group. PSP can be induced by ineffective male copulation which is consistent with probable neurological impairment in males. The neurotoxicity effects are already covered by the classification with STOT RE 1 (nervous system). In addition, substantial delays in PS and VO in rats are considered not substance-related *per se* but secondary to the considerably 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**.

Effects on development

The dataset consists of the following studies summarised in the Table below:

- Oral developmental toxicity study in rats (B.6.6.2, Study 1)
- Range finding oral developmental toxicity in rats (B.6.6.1, Study 1, tested up to 5 mg/kg bw/day, main study tested at higher doses)
- Dietary 2-generation study of reproductive toxicity in rats (B.6.6.1, study 2)

- Developmental neurotoxicity study in rats (B.6.7.3, Study 1)
- Range-finding oral developmental toxicity study in rabbits (B.6.6.2, Study 2a)
- Oral developmental toxicity study in rabbits (B.6.6.2, Study 2)

Table: *Most relevant studies for assessment of developmental effects*

Developmental toxicity studies		
Type of study; Reference	**Purity and dose levels of emamectin (base) compound**	**Observations**
Rat		
Oral developmental toxicity study (no guideline) Rat (CD), 25 mated females/dose B.6.6.2, Study 1	Purity: 94.2 % Orally by gavage; days 6-19 of gestation, 0, 2, 4, and 8 mg/kg bw per day	NOAEL: Maternal: 2.28 mg/kg bw/day Dev: 2.28 mg/kg bw/day LOAEL: Maternal: 4.56 mg/kg bw/day Dev: 4.56 mg/kg bw/day <u>Critical effects:</u> Maternal • ↑ bw gain (GD6-14: by 21 % at 4.56 mg/kg bw/day • ↑ incomplete ossification • ↑ supernumerary rib
Developmental neurotoxicity study Rat (CD) Oral range-finding reproduction study in female rats Rat Crl:CD(SD) Br strain 25 f/dose (no guideline, but in accordance with OECD TG 426) B.6.7.3, Study 1	Purity: > 97 % Orally by gavage, GD6-LD20 0, 0.1, 0.6, and 3.6/2.5* mg/kg bw/day *between gestation day 17 and 20 the high dose level of 3.6 mg/kg bw per day was reduced to 2.5 mg/kg bw per day due to the appearance of pup tremors in the 3.6 mg/kg bw/day dose group of a concurrent 2-generation reproduction study	NOAEL: Maternal: 2.85 mg/kg bw/day Dev: 0.68 mg/kg bw/day LOAEL: Maternal: > 2.85 mg/kg bw/day Dev: 2.85 mg/kg bw/day <u>Critical effects:</u> Maternal • No effects Development: • ↑ clinical signs of neurotoxicity • Growth retardation • Neurobehavioural effects
Rabbit		
Range finding - Oral developmental toxicity study (no guideline) Rabbit NZW, 10 pregnant females/dose* B.6.6.2, Study 2a *one female in the 4 mg/kg group was misdosed on GD 6, removed from the study and replaced by another female.	Purity: 96.2 % Orally by gavage; days 6-18 of gestation, 0, 2, 4, 6 and 8 mg/kg bw per day	NOAEL: Maternal: 4.56 mg/kg bw/day Dev: 9.12 mg/kg bw/day LOAEL: Maternal: 6.84 mg/kg bw/day Dev: > 9.12 mg/kg bw/day <u>Critical effects (top dose):</u> Maternal • ↓ bw gain (GD14-19) • ↓ food consumption (GD16 and GD22) Development (top dose): • One foetus showed cleft palate and hydrocephaly. • One foetus showed hydrocephaly.
Oral developmental toxicity study Rabbit NZW, 18 pregnant females/dose	Purity 94.2 % Orally by gavage; days 6-18 of gestation, 0, 1.5, 3, and 6 mg/kg bw per day	NOAEL: Maternal: 3.42 mg/kg bw/day Dev: 6.84 mg/kg bw/day LOAEL: Maternal: 6.84 mg/kg bw/day Dev: > 6.84 mg/kg bw/day

B.6.6.2, Study 2		Critical effects: Maternal • ↓ bw gain (by 40 %, GD6-19, by 52 % GD12-19) • ↓ food consumption (by 5 % max, ss on GD10, GD22) • ↑ clinical signs of neurotox: mydriasis, decreased pupillary reaction Development (top dose): • One foetus showed hydrocephaly

NZW: New Zeeland White; ss: statistically significant

Adverse effects on development in rats

Detailed results are presented in the background document (Supplemental information - In depth analyses by RAC).

In the main study (B.6.6.2, Study 1), maternal toxicity was observed in the top-dose group with clinical signs of neurotoxicity and a decrease in body weight gain. In the mid-dose group, the overall weight gain (days 0 through 20) was statistically significantly decreased by 5.2 %, and during days 14-20 by 13 %. In the top dose group (9.1 mg/kg bw/day), statistically significant decreases in body weight were seen during the complete gestational period (days 4-14; 14-20 and 6-20). Compared to control values, the overall reduction in body weight gain was 33 % and during GD14-20, this increased to 35 %. There was no evidence of treatment related pathology. The NOAEL for maternal toxicity and embryo/foetotoxicity was established at 2.28 mg/kg bw/day.

There was a dose-related increase in the number of foetuses with incomplete ossification and in the number of sites with incomplete ossification. An increase in the number of foetuses and in the number of sites with incomplete ossification was observed in the mid and high dose group. This effect was significant only in the high dose group. RAC concludes that this effect is possibly related to the minor decreased foetal weight and not a direct effect of emamectin benzoate on skeletal maturation. The foetal effects observed in the high dose group have not been correlated to the body weight effects in the dams.

RAC considers that the number of foetuses with skeletal variations in the high dose group is possibly treatment related. This was mainly due to increases in the number of wavy ribs and supernumerary ribs. RAC however notes that there is uncertainty surrounding the developmental/teratogenic significance of such ribs, in particular their post-natal reversibility. The increased incidence of supernumerary ribs is a relatively common finding in standard teratology bioassays, and previous studies have indicated a possible correlation between their occurrence and general maternal stress. The individual relationship between lower maternal body weight gain during treatment and the increase in supernumerary ribs was not reported. The supernumerary ribs in the rat may be considered as a result of developmental delays in a labile region of the axial skeleton and not as a manifestation of a teratogenic event. HCD from more than 200 studies conducted before or during the same time period (Lang, 1993) show that wavy ribs and supernumerary ribs incidences in the present study are above the average but below the maximum incidence observed.

Both pre- and post-implantation losses were increased in the top dose group relative to concurrent controls but no historical control data was available. A slight decrease in foetal weight

and an increase in the number of resorptions were observed in the high dose group. The number of resorptions in the high dose group was increased compared to the controls. This was partly due to 2 litters with 4 and 6 resorptions. The increase was considered to be within normal biological variation according to the study authors, since the percentage of resorptions plus dead foetuses per implants (4.3 % for the high dose group) was close to the mean value (4.0 %) of historical control groups from recently performed studies. RAC considers the increase of the number of resorptions and post-implantation losses in the high dose group as possibly treatment-related. RAC further notes that the two females with 2 litters with 4 and 6 resorptions did not present more pronounced decreases in body weight gain or food consumption compared to other treated females. Therefore, there is some doubt as to whether these changes could simply be explained by excessive maternal toxicity. RAC further notes that there was no effect on pre- or post-implantation losses and resorptions in either the rabbit dose-range finding study or developmental main study.

In addition to the above noted variations, there was one pup with cleft palate, this finding needs to be carefully considered for classification and labelling since the female dam had normal body weight gain and food consumption. In addition, no clinical signs were noted over the gestation period. Cleft palate is a very rare malformation in rats though single spontaneous occurrences can be observed. The same malformation was also observed in a dose-range finding study in rabbits (see below) as well as with the similar substances abamectin (RAC, 2010) and ivermectin (Wise *et al*., 1997). No HCD were available from the performing laboratory for these malformations. A few examples of rarely occurring malformations in Crl:CD (SD) rat foetuses and rabbit foetuses are given in the textbook in Hood (2012). Among more than 59 000 rat foetuses in the authors' HCD, cleft lip/palate accounts for 0.01 % (5/59 744 foetuses; 165 rat developmental toxicity studies or 1 foetal incidence in 33 studies; 1998-2010). Among more than 12 000 rabbit foetuses, cleft lip/palate accounts for 0.05 % (6/12 222 foetuses; 69 rabbit developmental toxicity studies or 1 incidence per 11.5 studies, 2006-2009).

Adverse effects on development in rabbits

Detailed results are presented in the background document (Supplemental information - In depth analyses by RAC).

In the dose-range finding study, two foetuses from two separate litters in the high dose group had malformations (one foetus had cleft palate and hydrocephaly and one foetus had hydrocephaly only). The study authors reported an incidence of 0.04 % for the testing laboratory HCD for rabbit cleft palate and that no such HCD existed for hydrocephaly. RAC notes that these affected foetuses were from dams which showed the greatest body weight losses during the dosing period and/or had tremors so the malformations were present with concomitant significant maternal toxicity. HCD for hydrocephaly from the conducting laboratory were not available within the study reports. RAC notes that late in the opinion process industry supplied further data in support of the rabbit developmental findings. The recorded incidences of hydrocephaly in the emamectin study were within the HCD range in the New Zealand White rabbit, from the same breeder between 1984 and 1990. The highest foetal/litter incidence of spontaneously occurring hydrocephaly recorded during 1984-1990 was 3 foetuses (2.7 %) from 2 litters (11.8 %). In the main rabbit study, one case of hydrocephalus was reported at the top dose. The doe did not show maternal neurotoxicity or excessive decreased body weight gain. However, the case of hydrocephaly was also reported at the highest dose only (i.e. with a threshold and no dose-response). RAC further notes that a previously reported HCD for hydrocephaly was up to 0.97 % (mean; range: 0-8.3) in the same strain and during the same period so the incidence from the main rabbit study (0.7 %) lies within these HCD (RAC, 2017).

For cleft palate which is also a very rare malformation, RAC notes that a Japanese publication reported incidences of 0.14 % (0-0.9) and 0.05 % (0-0.60) (external anomalies in New Zealand White rabbits during the study period of 1994-2000) but higher and more variable values during 2001-2010 (0.03 % (0-1.96), 0.02 % (0-0.61) and 0.16 % (0-2.00). While Hood (2012) gives an incidence of 0.05 % (6/12 222 foetuses; 69 rabbit developmental toxicity studies; 2006-2009).

Developmental neurotoxicity in rats

The DS reported the results of a developmental neurotoxicity study with rats given emamectin benzoate by gavage. The study is summarised in details in Annex 1 to the CLH report as well as in the publication by Wise *et al*., 1997. Detailed results are presented in the background document (Supplemental information – In depth analyses by RAC).

The NOAEL for developmental neurotoxicity was established as 0.68 mg/kg bw/day, based on the occurrence of clinical evidence of neurotoxicity, growth retardation and alterations of neurobehavioural function in the F1 progeny of females administered emamectin benzoate hydrate at 2.85 mg/kg bw per day during the period of gestation (day 6) through lactation (day 20). In the pups, no histopathological evidence of neurotoxicity was observed. In the absence of evidence of toxic effects in dams, the NOAEL for maternal toxicity was 2.85 mg/kg bw/day, expressed as emamectin benzoate, the highest dose tested.

For abamectin, acting via a similar mode of action, RAC concludes that pup mortality due to exposure at 0.4 mg/kg bw/day (significant in F1A, F1B and F2A pups) is likely to be the result of a reduced p-glycoprotein expression in the neonatal rat brain and a particularly high susceptibility of the offspring (RAC, 2010). The higher sensitivity of neonates (as well as CF-1 mice) to avermectins, which are known substrates for P-glycoprotein and mediated by the expression and functionality of the P-glycoprotein drug efflux transporter, should be considered with caution. P-glycoprotein is a key component of the blood-brain barrier. Therefore, the RAC opinion disregarded neonate toxicity as relevant for classification (RAC, 2010).

The applicant conducted a single dose oral kinetic study in wild type (+/+) and p-glycoprotein mutant (-/-) CF-1 mice to investigate the concentrations of radiolabelled ivermectin, abamectin and emamectin benzoate in brain and plasma (see the DAR 2008, section B.6.8.2, kinetic study in genotyped CF-1 mice). The results showed that brain concentrations of emamectin benzoate were about 150-fold higher in p-glycoprotein mutant (-/-) mice compared with (+/+) mice, and that the differences observed were comparable to those seen for both abamectin and ivermectin. These results provide good indirect evidence that emamectin benzoate, as expected, has similar P-glycoprotein substrate specificity to that of ivermectin and abamectin. The conclusions on abamectin with regard to the relevance of the sensitive CF-1 mouse are therefore also applicable to emamectin benzoate.

RAC acknowledges that in contrast to rats, humans are born at a more advanced stage of overall development. This relatively greater developmental maturity is also reflected in a much better developed blood brain barrier in the human neonate compared with rat. Recently, Lam *et al*. (2015) examined P-glycoprotein levels across the human foetal, neonatal and adult periods; they showed brain P-glycoprotein levels of 35 % of adult at 20-26 weeks of gestation, 43 % of adult by weeks 36-40 of gestation, and 58 % of adult at 0-3 months of age.

RAC notes however that Lam *et al*. (2015) reported that neonates and young infants may be more sensitive to the central depressive effects of various xenobiotics, e.g. morphine, compared to older children and adults in both animal and human studies. In rats and mice, the brain P-glycoprotein expression and function matures at 21 days. The numerous and well-aligned datasets in mice and rats contrast with very few and conflicting reports in non-human primates. RAC stresses that it is currently difficult, with the existing data, to determine quantitative and qualitative differences to humans, especially in refining the maturation time frame of a critical

drug efflux transporter. In addition, there are other mechanisms for a possible increased neonatal sensitivity to avermectins such as slower clearance rate, enhanced intestinal absorption, and disrupted or higher brain blood barrier permeability due to other mechanisms. RAC notes that these mechanisms have not been explored with emamectin benzoate and that there are no data in non-human primates.

Conclusion and comparison with the CLP criteria

Regarding developmental neurotoxicity, RAC notes that emamectin benzoate caused clinical evidence of neurotoxicity (clinical signs), growth retardation (as evidenced by decreased pup body weight and body weight gain, delay in ossification) and alterations of neurobehavioural function effects in pups from mothers exposed to emamectin benzoate. RAC recognises the high susceptibility of these pups to the substance as well as the structurally similar substances abamectin and ivermectin. Neonatal rats are however known to have a limited expression of P-glycoprotein until about 5 weeks of age and, of equal if not more importance, an incomplete development of the blood-brain barrier both before and after birth (Lankas *et al.*, 1989, Matsuoka *et al.*, 1999; Betz and Goldstein, 1981; JMPR, 2011; EFSA, 2007; EFSA, 2012). Therefore, although no clear increased sensitivity to emamectin benzoate 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. The neurotoxicological effects in the F1 offspring were observed only in the highest dose level (2.85 mg/kg/day). As noted above, these effects were observed in conjunction with moderate to marked decreases in pre-weaning weights, post-weaning weight gains, and delays in attainment of developmental landmarks. A NOAEL for developmental neurotoxicity was determined to be 0.68 mg/kg/day i.e. above the doses causing neurotoxicity in adults. In view of these uncertainties, RAC prefers to follow the RAC opinion for abamectin (2010) and concludes that the effects are covered under STOT RE 1 and are therefore not considered for classification under developmental toxicity.

For the effects observed in developmental toxicity studies in rats and rabbits, there is no information on the potential of emamectin benzoate to adversely affect development in humans and therefore classification in Category 1A is not warranted. RAC also concludes that the whole data package available for emamectin benzoate does not provide clear evidence of adverse effects on development therefore Category 1B is not appropriate.

Regarding classification in category 2 (suspected human reproductive toxicant), there is some doubt as to whether some effects could simply be explained by excessive maternal toxicity or be spontaneous. RAC considers the fact that cleft palates were also reported for similar substances i.e. abamectin (RAC, 2010) and ivermectin (Wise *et al*., 1997) to increase the concern for these effects. However, RAC must primarily focus on this particular substance and deal with its specific data package with respect to proposing classification. Other effects of concern include resorptions in rats as well as delayed ossification and increased variations. Therefore, the weight of evidence is borderline to support the classification of emamectin benzoate for developmental toxicity (cat. 2).

The increased number of very rare malformations in both rats (cleft palate, single incidence) and rabbits (one cleft palate and two hydrocephaly; notwithstanding they were observed in does with the highest maternal toxicity), though small, are of concern to RAC though it must be recognised single cases may sometimes still appear in both species. No robust HCD was available for hydrocephaly from the performing laboratory but some data from another facility using the same animal stock was made available. The findings in the rabbit dose-range finding study are compromised by considerable toxicity in the two affected females, and as the top dose was decreased in the full study, the findings (for cleft palate) could not be reproduced. This lowering of the dose level in the main study (and therefore maternal neurotoxicity) could account for the

absence of cleft palate. However, one case of hydrocephaly was found in the full rabbit study but this could also be sporadic in nature. RAC notes that late in the opinion process industry supplied further data in support of the rabbit developmental findings. HCD for hydrocephaly in the New Zealand White rabbit was sourced from the same breeder colony as the emamectin studies. This data clarified the spontaneous incidence of hydrocephaly in this strain and colony of rabbit, and RAC took note of this data derived from a different facility for two reasons: (1) The appearance of hydrocephaly is distinctive and not open to different interpretation between laboratories, (2) the same breeder supplied the laboratories for both the emamectin studies and those contributing to the HCD in industry's paper "Hydrocephaly – Historical Control Data to Support Prenatal Developmental Toxicity Studies in the New Zealand White Rabbit".

The recorded incidences of hydrocephaly in the emamectin study were within the HCD range in the New Zealand White rabbit, from the same breeder between 1984 and 1990. The highest foetal/litter incidence of spontaneously occurring hydrocephaly recorded during 1984-1990 was 3 foetuses (2.7 %) from 2 litters (11.8 %).

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) (RAC, 2010). 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 emamectin benzoate does not warrant classification for developmental toxicity**.

Adverse effects on or via lactation

There is evidence that the analogue ivermectin is found in high concentrations in milk, resulting in higher exposure of offspring than of the dams. Wise *et al*. (1997) reported that in multigeneration studies in rats conducted with ivermectin and abamectin, postnatal toxicity was characterized by decreased weight gain and mortality in F1 offspring at doses of 0.4 mg/kg bw/day for both compounds. Further studies using radiolabeled ivermectin indicated that high drug concentrations in the milk of exposed dams (3- to 4-fold higher than in maternal plasma) led to high drug levels in the plasma and brain of the F1 offspring (relative to adult rats), leading to toxicity.

That emamectin is toxic to offspring is also clear from the available database. RAC notes that in the rat oral (gavage) range-finding reproduction study emamectin benzoate (B.6.6.1, Study 1), administered to dams at the top dose of 5 mg/kg bw/day was highly foetotoxic up to LD 14. There were treatment related increases in the percent pup deaths. The top dose group was euthanised early between LD 8-15. There were no treatment related deaths in the other dose groups. Severe reductions in mean pup body weight (52 % below controls by PND 14) was also noted. Clinical signs and nervous system pathology were not reported for pups from the top dose gavage group. Clinical signs and nervous system pathology were remarkable for pups from the top dose (50 ppm) dietary section of this study but there were no pup deaths in any of the dietary groups. Results of animal metabolism studies in lactating goats (Anonymous, 1995) demonstrated the presence of emamectin benzoate in milk (12-56 ppb), but only modestly higher than concentrations found in the plasma (8-38 ppb). The physical-chemical properties of ivermectin and emamectin are similar, and with a log K_{ow} of 5 at pH 7 for emamectin, transport into milk is highly likely. Direct evidence that emamectin is found in high concentrations in milk is however lacking.

RAC notes that rodent offspring are particularly sensitive to the neurotoxicity of emamectin benzoate, primarily during exposure via lactation. However, since specific data for content of emamectin benzoate in milk is lacking, RAC considers the case for classification via lactation as inconclusive.

RAC also notes that not enough is known about the relative differences in neurotoxicity of emamectin benzoate in juveniles. Thus, the toxicity seen in the neonatal rat during lactation may not be representative of potential toxicity in a neonatal human. **RAC supports no classification for lactation** but stresses that the difference in susceptibility of neurotoxic effects seen in pre-weaning rats by emamectin benzoate remains questionable.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Emamectin benzoate consists of emamectin B1a benzoate and emamectin B1b benzoate and is not currently included in Annex VI to the CLP Regulation. The DS proposed to classify the substance as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410. The substance is not rapidly degradable and has a low potential for bioaccumulation. The lowest acute toxicity value of 0.000040 mg/L for *Mysidopsis bahia* warranted an M-factor of 10 000 and the lowest chronic toxicity value of 0.000088 mg/L for *Daphnia magna* for a non-rapidly degradable substance warranted an M-factor of 1 000. After the consultation, the DS changed the chronic classification proposal to Aquatic Chronic 1, M-factor of 10 000 based on the use of the surrogate approach for the acute *Mysidopsis bahia* data.

Degradation

There was one ready biodegradability test available on emamectin benzoate. The test was performed according to OECD TG 301F. Biodegradation of emamectin benzoate B1a was 0 % after 28 days. The DS concluded that emamectin benzoate was not readily biodegradable.

In the only hydrolysis study available, emamectin B1a was tested at different pH (pH 5, 6, 7, 8, and 9) and temperatures. The study was performed following the OECD TG 111 and GLP. Emamectin B1a was hydrolytically stable at environmentally relevant pH (4-8) and temperature (25 °C). Under basic conditions (pH 9), the DT_{50} for hydrolysis of emamectin B1a was 19.5 weeks at 25 °C. Two unidentified degradation products were formed at 9.1 and 9.9 % of AR (Applied Radioactivity).

One of the two laboratory water/sediment degradation studies available was accepted for degradation rate derivation. In the study performed according to the OECD TG 308, a silt loam system and a sand system were applied with [23–^{14}C]-emamectin benzoate B1a and incubated under aerobic conditions at 20 °C in the dark. The DT_{50} values obtained for emamectin benzoate B1a were:

- $DT_{50,water}$ 8.7 days for both systems, dissipation rate
- $DT_{50,system}$ > 120 days

The decline of concentrations in the water phase was mainly caused by a rapid initial sorption, and the $DT_{50water}$ thus represented dissipation rather than degradation. The maximum level of emamectin benzoate B1a found in sediment was 71.3 and 83.0 % of AR after 90 and 120 days, respectively. Bound residues increased to 20.2-10.7 % of AR at the end of the study. Mineralisation was low with a maximum of 1.4 % of AR after 21 days in the silt loam system.

Observed metabolites were not observed in significant quantities and were therefore not identified.

The photodegradation of emamectin B1a was determined in three studies. The DT_{50} values for photolysis ranged from 0.5 days to 65 days. The lowest values were reached in sensitised conditions. Several metabolites were identified in low amounts (< 10 %). The highest metabolite amount was 18.3 %.

Based on the information presented, the DS concluded that emamectin benzoate was not rapidly degradable for classification purposes.

Bioaccumulation

The bioaccumulation of emamectin benzoate B1a was studied in *Lepomis macrochirus* according to US EPA 54019-82-021 and ASTM E1022-84 guidelines. The mean measured radioactivity concentration in the treatment chambers during the 28-day exposure phase, was equivalent to 1.2 ± 0.095 µg 3H-MAB1a/L. Test criteria were not completely met since the experiment did not last long enough to reach three consecutive samples in the steady state. For the 21-28 days interval, the recalculated mean BCFs were 30, 102 and 82 L/kg wwt for edible tissue, nonedible tissue and whole fish, respectively. The kinetic whole fish BCF was reported to be 80 L/kg wwt, which was confirmed by the DS. The BCF values were not corrected for growth or normalised to 5 % lipid content and there was no data available for the DS to make the normalisation. The DS concluded that while there remained some uncertainty with regard to the bioaccumulation potential of emamectin benzoate B1a, the experimental data did suggest a low bioaccumulation potential.

The log K_{ow} for emamectin benzoate depended on pH and was determined to be 5.9, 5.0 and 3.0 at pH 9.0, 7.0, and 5.0, respectively. The methodology had not been specified, and study details were not available. Considering the experimentally determined pK_a of 7.7 and the fact that the molecule was increasingly neutrally charged at higher pH values, these log K_{ow} values correspond with what might be expected. Furthermore, QSAR estimated values are in the same range, i.e. a log K_{ow} of 2.93 for the ionic species, and a log K_{ow} of 6.17 for the neutral species.[1] Initially, the BCF values and log K_{ow} values appear conflicting, with the BCF of 80 L/kg wwt suggesting low bioaccumulation potential and the log K_{ow} of 5.9 indicating bioaccumulation potential. The DS also assessed the size and dimensions of the molecule itself. As stated in REACH guidance R.11 a molecule with an average maximum diameter of greater than 1.7 nm plus a molecular weight of greater than 1 100 may be considered as not bioaccumulative in PBT assessment. Emamectin is a large molecule with a diameter of 2.1 nM and a molecular size of 1 008.3.

Based on the evidence available and using the BCF as primary evidence, the DS concluded that while the log K_{ow} of the neutral molecule was above the CLP criterion, emamectin was considered to have a low bioaccumulation potential.

[1] For emamectin benzoate two dissociation constants are found: a pK_a of 7.7 for the epimethylamino part of the emamectin ion ($(R_2\text{-}NH_2^+$; conjugated acid) and a pK_b of 9.8 for the benzoate ion (conjugated base), which corresponds to a pK_a of 4.2 for benzoic acid.

Aquatic toxicity

***Table**: Reliable aquatic toxicity data on emamectin benzoate*

Test material	**Method** (including test species)	**Acute results, mg/L**	**Chronic results, mg/L**	**Reference**
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S002, purity 95.9 %	ASTM E 729-88; EPA 540/9-82-024 *Oncorhynchus mykiss* flow-through methanol used as solvent (1	96 h LC_{50} = 0.174 mm 68-123 % of nominal		STUDY IIA 8.2.1.1/01
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S002, purity 95.9 %	ASTM E 729-88; EPA 540/9-82-024 *Lepomis macrochirus* semi-static methanol used as solvent	96h LC_{50} = 0.180 mm 88-112 % of nominal		STUDY IIA 8.2.1.2/01
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 94.6 %, appearance white powder Radiolabelled MK-244, batch L-683,825-055J006, 15994-111/95-137, purity 99.3 %	ASTM E 729-88; EPA 540/9-82-024 *Pimephales promelas* flow-through methanol used as solvent (1	96h LC_{50} = 0.194 mm 69-87 % of nominal		STUDY IIA 8.2.1.2/02
Technical MK-244 (emamectin benzoate), batch L-656, 748-052 S005, purity 94.6 %; 3HMK244, batch [3H] L-683, 825-005J006, ([5-3H] epimethylamino-avermectin B1a benzoate) and L-683,825-005J006, ([5-3H] epimethylamino-avermectin B1a benzoate), substances suspended in ethanol	ASTM E1241-88, 1988 US EPA 540/9-82-024, 1982 and 540/9-86-138, 1986 *Pimephales promelas* flow-through methanol used as solvent (1		32d NOEC = 0.012 mm 79-93 % of nominal (length, wet and dry weight)	STUDY IIA 8.2.4/01
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 95.9 %	ASTM E 729-88; EPA 540/9-82-024 *Cyprinodon variegatus* salt water flow-through methanol used as solvent (1	92 h LC_{50} = 1.430 mm 83-109 % of nominal		STUDY IIA 8.2.1.2/04

Test material	**Method** (including test species)	**Acute results, mg/L**	**Chronic results, mg/L**	**Reference**
MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 95.9 %	US EPA 540/9-82-024; ASTM E 729-88 *Daphnia magna* flow-through methanol used as solvent (1	48 h EC_{50} = 0.001 (immobility, mortality) mm 58-67 % of nominal		STUDY IIA 8.3.1.1/01
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S-002, purity 97.5 %: Radiolabelled MAB1a ([3H]MK-244), batch L-653,825-055J001, 15670-101-28/93-325	US EPA 540/9-82-024; ASTM E 1193-87; US EPA 540/9-86-141 *Daphnia magna* flow-through methanol used as solvent (1		21d NOEC 0.000088 (survival) mm 80-88 % of nominal	STUDY IIA 8.3.2/001
Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 95.9 %	ASTM E 729-888, EPA 540/9-82-024 *Crassostrea virginica* flow-through methanol used as solvent (1	96 h EC_{50} = 0.530 (shell deposition) mm 76-120 % of nominal		STUDY IIA 8.3.1.1/03
MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 95.9 %; 3HMAB1a (emamectin B1a), batch L683,825-001A009; 18075-148; 93-014, radiochemical purity 97.2 %	EPA 540/9-82-024, EPA540/9-85-010, ASTM E 729-88 *Mysidopsis bahia* salt water flow-through methanol used as solvent (1	96 h LC_{50} = 0.00004 mm 54-85 % of nominal		STUDY IIA 8.3.1.1/04
MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 94.6 %, appearance white powder; 3H-MK-244, batch L-683,825-005J006	US EPA 540/9-82-020 *Pseudokirchneriella subcapitata* static dimethylformamide used as solvent (1	120 h E_rC_{50} ≥ 0.0039 Initial concentration, only one concentration tested. pH 7.4-9.6	120 h NOEC ≥ 0.0039 Initial concentration, only one concentration tested. pH 7.4-9.6	STUDY IIA 8.4/001

Test material	Method (including test species)	Acute results, mg/L	Chronic results, mg/L	Reference
MK-244 (emamectin benzoate), batch SSH2F004, purity 97.3 %	US EPA OPPTS 850.5400; OECD 201; EC, L383 A, Part C.3 *Pseudokirchneriella subcapitata* static solvent use not mentioned	96 h E_bC_{50} = 0.0072 96h E_rC_{50} = 0.0121 mm below LOQ – 55 % of nominal pH 7.3-9.7	NOEC < 0.0046 mm below LOQ – 55 % of nominal pH 7.3-9.7	STUDY IIA 8.4/002
MK-244 (emamectin benzoate), batch L656,748-052S005, purity 94.6 %; 3H-MK-244 batch nr. L-683,825-005J006, purity 99.3 %	US-EPA 540/9-82-020; ASTM E 1415-91 *Lemna gibba* static-renewal dimethylformamide used as solvent *	14 d IC_{50} > 0.094 mm 62-85 % of nominal (fresh solutions)		STUDY IIA 8.6/01

* The calculated water solubility of the substance: 0.32-0.024-0.0001 g/L at pH 5, 7, 9.
mm = based on mean measured concentrations
LOQ = Limit of quantification

Acute Aquatic Toxicity

There are four reliable acute fish toxicity studies available. The lowest acute toxicity value for fish was a 96 h LC_{50} of 0.174 mg/L for *Oncorhynchus mykiss.*

For invertebrates, there are reliable studies available on *Daphnia magna*, *Crassostrea virginica* and *Mysidopsis bahia*. The lowest acute toxicity value was a 96 h LC_{50} of 0.00004 mg/L for *Mysidopsis bahia*.

There are two reliable studies available for algae and one for *Lemna minor*. The lowest acute toxicity value was a 96 h E_rC_{50} of 0.0121 mg/L for *Pseudokirchneriella subcapitata.*

The lowest acute toxicity value for emamectin benzoate is a 96 h LC_{50} of 0.00004 mg/L for *Mysidopsis bahia*. The study was a guideline flow-through study following GLP. Mean measured concentrations were 7.8, 18, 26, 41 and 72 ng/L. At day 0, substance recovery ranged from 54 to 71 % of nominal values. At 96 h recovery ranged from 65 to 85 % of nominal values. No control mortality or mortality occurred in the solvent control at 7.8 and 18 ng/L. Mortality of 10, 45 and 100 % was observed at 26, 41 and 72 ng/L.

Chronic Aquatic Toxicity

There were reliable chronic toxicity data available on fish, Daphnia and algae. The lowest chronic toxicity value was a 21 d NOEC (survival) of 0.000088 mg/L for *Daphnia magna.* The study was a guideline flow-through study following GLP. Nominal test concentrations were 0.050, 0.10, 0.20, 0.40, 0.80 µg/L. Mean measured concentrations during the test ranged from 80 to 88 % of nominal values (0.043, 0.088, 0.16, 0.34 and 0.67 µg/L). The reproduction results of the solvent control differed significantly from the negative control. Therefore, the solvent control was used

for comparisons among the treatment groups. Reproduction at 0.043 and 0.088 µg/L did not significantly differ from the solvent control.

Comments received during the consultation

Two Member States (MS) agreed with the proposed classification. One MS felt that the surrogate approach should be used for chronic classification because there was no chronic data available for the acutely most sensitive species *Mysiodopsis bahia*. This approach would indicate classification as Aquatic Chronic 1, M-factor of 10 000. The DS agreed with this approach and changed their chronic classification proposal accordingly following the approach outlined in the CLP guidance (Section 4.1.3.3.1).

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider emamectin benzoate as a not rapidly degradable substance:

- the biodegradation of the substance in the OECD TG 301 F test was 0 % after 28 days showing that the substance is not readily biodegradable.
- in an OECD TG 308 water/sediment degradation test the dissipation rate $DT_{50,water}$ was 8.7 days for both systems and the $DT_{50,system}$ was > 120 days showing rapid initial sorption to the sediment. The maximum level of emamectin benzoate B1a found in sediment was 71.3 and 83.0 % of AR after 90 and 120 days, whilst mineralisation was low. The observed metabolites were not major and therefore not identified. This shows that the substance is not ultimately degraded in a surface water simulation test with a half-life of < 16 days.
- In the OECD TG 111 hydrolysis study, Emamectin B1a was hydrolytically stable at environmentally relevant pH (4-8) and temperature (25 °C). Under basic conditions (pH 9), the DT_{50} was 19.5 weeks at 25 °C. Two unidentified degradation products were formed at 9.1 and 9.9 % of AR (Applied Radioactivity). Thus, it is not demonstrated that the substance is primarily degraded biotically or abiotically e.g. via hydrolysis, in the aquatic environment with a half-life < 16 days.

Bioaccumulation

The BCF for *Lepomis macrochirus* for the 21-28 days interval (plateau levels chosen by the RMS) was 82 L/kg wwt for the whole fish. The corresponding kinetic BCF was 80 L/kg wwt. BCF based on total radioactivity, transformation may have taken place and values are worst case. The BCF values were not corrected for growth or normalised to 5 % lipid content. Considering that the experimental BCF values are far from the classification cut-off of 500, this these shortcomings are not decisive.

The log K_{ow} for emamectin benzoate depended on pH and was determined to be 5.9, 5.0 and 3.0 at pH 9.0, 7.0, and 5.0, respectively. The OECD TG 107 (Shake-flask-method) is normally not applicable for surface-active substances. The good repeatability together with the good recoveries in this test, however, showed that the surface activity of emamectin benzoate did not influence the results.

Based on the evidence presented, RAC agrees with the DS to conclude that emamectin benzoate has a low bioaccumulation potential.

Aquatic toxicity

There were reliable acute toxicity data available for fish, invertebrates, algae and *Lemna*. The lowest reliable acute toxicity value for emamectin benzoate was the 96 h LC_{50} of 0.00004 mg/L for *Mysidopsis bahia*. In conclusion, RAC agrees with the DS that emamectin benzoate warrants classification as Aquatic Acute 1; H400, M=10 000 ($0.00001 < L(E)C_{50} \leq 0.0001$).

There were chronic toxicity data available for fish, invertebrates and algae. The lowest chronic toxicity value for emamectin benzoate was a 21 d NOEC for survival of 0.000088 mg/L for *Daphnia magna.* The lowest chronic toxicity value is 0.000088 mg/L for Daphnia magna which is in the range of $0.00001 < NOEC \leq 0.0001$, giving an M-factor of 1 000. As the surrogate approach using the acute data for *Mysidopsis bahia* results in a more stringent outcome, this is used for classification. However, RAC agrees with the DS's proposal amended after the consultation to use the surrogate method for chronic classification, which changed the proposed M-factor from 1 000 to 10 000. The substance is not rapidly degradable and has a low potential for bioaccumulation. The lowest acute toxicity value is 0.00004 mg/L for *Mysidopsis bahia*, which is in the range of $0.00001 < L(EC)_{50} \leq 0.0001$, giving an M-factor of 10 000.

RAC notes that as emamectin benzoate is an insecticidal substance, the classification may need to be reconsidered if additional aquatic insect toxicity data (e.g. Mayfly) becomes available in the future.

In conclusion, RAC agrees with the DS's proposal (amended after the consultation) to classify emamectin benzoate as **Aquatic Acute 1; H400, M=10 000 and Aquatic Chronic 1; H410, M=10 000**.

Additional references

Betz, A. L. and Goldstein, G. W. (1981): Developmental changes in metabolism and transport properties of capillaries isolated from rat brain, J. Physiol., 312: 365-376.

Developmental and Reproductive Toxicology: A Practical Approach, 3rd ed. RD Hood. 2012

EFSA (2012). Conclusion on the peer review of the pesticide risk assessment of the active substance emamectin. EFSA Journal 2012; 10(11): 2955. https://www.efsa.europa.eu/fr/efsajournal/pub/2955 (accessed in July 2019).

Hafez, E.S.E. (1970); Book: Reproduction and Breeding Techniques for Laboratory Animals. Pub. Lea and Febiger, PA, US.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2011). http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report11/Emamectin.pdf (accessed in July 2019).

Lang, 1993. Historical Control Data for Development and Reproductive Toxicity Studies using the CrI:CD®BR Rat. Compiled by MARTA (Middle Atlantic Reproduction and Teratology Association). Edited by Patricia L. Lang, Ph.D., Consultant in Toxicology, September 1993.

Lankas G.R., Minsker D.H. and Robertson R.T. (1989); Effects of ivermectin on reproduction and neonatal toxicity in rats. Fd. Chem. Toxic. 27(8):523-529.

Matsuoka Y., Okazaki M., Kitamura, Y., and Taniguchi T. (1999). Developmental expression of P-glycoprotein (multidrug resistance gene product) in the rat brain. Journal of Neurobiology, Vol. 39 (3), 383-392.

Anonymous, 1995. The elimination, tissue distribution and metabolism of [3H]/4”-deoxy-4”-epimethylaminoavermectin B1a (MAB1a) benzoate and [3H/14C] MAB1a benzoate in lactating goats. GLP, not published.

Ogbuokiri J. E., Ozumba B. C. and Okonkwo, P.O. 1993. Ivermectin levels in human breast milk. Eur. J. Clin. Pharmacol. 45, 389-390.

Sulik K.K., Cook C.S., and Webster W.S. 1988. Teratogens and craniofacial malformations: relationships to cell death. Development 103 (Suppl), 213–231.

Stevens J., Breckenridge C., and Wright J. 2010. The Role of P-glycoprotein in Preventing Developmental and Neurotoxicity: Avermectins – A Case Study. Hayes’ Handbook of Pesticide Toxicology, Chapter 97, 2093-2110.

Wise L.D., Allen H.L., Hoe C-M. L., Verbeke D.R. and Gerson R.J. 1997. Developmental Neurotoxicity Evaluation of the Avermectin Pesticide, Emamectin Benzoate, in Sprague–Dawley Rats. Neurotoxicology and Teratology, Vol. 19, No. 4, pp. 315–326, 1997.

Wrzesinski C.L., Feeney W.P., Feely W.F., Crouch L.S. Dermal penetration of 4"-(epi-methylamino)-4"-deoxyavermectin B1a benzoate in the rhesus monkey. Food Chem Toxicol. 35(10-11):1085-9.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in ‘RAC boxes’.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential documents).