ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPSAL ON ABAMECTIN AND AVERMECTIN B1A

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Substance name: abamectin
CAS number: 71751-41-2

Substance name: avermectin B_{1a}
CAS number: 65195-55-3
EC number: 265-610-3

General comments

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 49 We support to establish a harmonised classification & labelling for abamectin, which is an active ingredient in plant protection products (Dir. 91/414/EEC) and biocide products (Dir. 98/8/EC).	Thank you for your support	The support is noted.
2009/10/15	United Kingdom / Audrey Pearson / MSCA	We agree with the environmental classification and labelling proposal overall, but further interpretation of fate data is required along with clearer justification for the use of ecotoxicity data using a 'non-standard' species that appears to be significantly more sensitive than other aquatic species (since this is the basis for the very large M-factor). • The spelling of abamectin varies throughout (e.g. abamectin versus abamectine) and should be consistent. • The document states that the variation	Thank you for your support. We would like to thank the UK for their detailed editorial comments. We agree with most of them and revised the background document accordingly. Regarding your remark on the justification for the use of ecotoxicity data using a 'non-standard' species. According to the CLP both freshwater and marine species toxicity data are considered suitable for use in classification provided the test method equivalent to standardised	Agree with MS reply

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		in purity is not expected to substantially affect fate/behaviour/ecotoxicity and therefore the classification and labelling. It would be helpful to present more detailed discussion and comparison of the relevant data to support this argument. For example, where studies were conducted on a single component such as B_{1a}, how does this relate to B_{1b}?	test methods. This justification is added.	
2009/10/16	France / Antony Fastier / AFSSA	The entire series of avermectins seems to share a common mode of action: to increase membrane permeability and to act as GABA agonists. According to the results obtained in the toxicological studies with abamectin, the proposed labeling should be : T+, R26/28 R48/23/25 Repr. Carc. cat.3 R63 N, R50/53 However, we propose to remove the classification Repr. Carc. Cat.3 R63. In the reproductive toxicity studies with abamectin, pup malformations, which were considered not secondary to maternal toxicity, and increase in post-natal mortality, which was most likely an effect on or via lactation, were observed. It was already shown that increased sensitivity for avermectin toxicity is	The classification proposal is based on malformations observed in developmental toxicity studies in rats and rabbits. It is acknowledged that differences in p-glycoprotein expression in the developing brain occur between humans and rats, explaining the neurotoxicity observed during lactation of newborn rats.	The classification and labelling proposal is mainly based on the rabbit data. It is known that P-glycoprotein is present in adult rabbits, whereas there is no data on the presence, or lack of, P-glycoprotein in fetal rabbits. It is therefore prudent to assume that toxicity data from rabbits can be of relevance

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		related to a reduced P-glycoprotein expression. This was demonstrated for CF-1 mice and neonatal rats : - CF-1 mice have reduced P-glycoprotein expression and increased sensitivity for avermectin toxicity compared to CD-1 mice - Due to neonatal rats having limited P-glycoprotein expression until 20 days after birth, they have an increased susceptibility for avermectin toxicity. P-glycoprotein dependent xenobiotic efflux in the blood brain barrier and placental mother/fetus barrier play an important role in attenuating the known neurotoxicity of avermectins and the developmental toxicity of ivermectin and abamectin. This protein contributes to three layers of protection : - limiting absorption of xenobiotics from the gut, - removing xenobiotics from the blood by excretion via bile and urine, - protecting the foetus and vulnerable organs such as the brain through its role in barrier epithelia. P-glycoprotein genes are found in all animals and are particularly highly conserved in mammals and humans. It could be assumed that the toxicological effects observed with avermectins are not	However, in the dams in the developmental toxicity studies the p-glycoprotein is expressed, thus it can be assumed that, similar to humans, absorption of abamectin from the gut will be limited and abamectin will be excreted in the bile in these animals. Furthermore, pgp is also expressed in rodent placenta (Ting Wang, Man Chen, You-e Yan, Feng-qin Xiao, Xiao-liang Pan, Hui Wang, Growth Retardation of Fetal Rats Exposed to Nicotine In Utero: Possible Involvement of CYP1A1, CYP2E1, and P-Glycoprotein, Environ Toxicol. 2009 Feb;24(1):33-42.) (and presumably also in rabbit placenta), thus reducing fetal exposure. The data suggest that abamectin may induce malformations during fetal development, indicating that the pgp in the placenta is not capable of adequately preventing fetal exposure during (certain periods of) the gestation. It cannot be assessed whether the human fetus is better protected from abamectin exposure than rat or rabbit fetuses. In view of this, it is assumed that the malformations may also be relevant to humans, and that the classification Repr. Carc. Cat.3, R63 is justified.	for humans. Furthermore, it is not known when the club-foot malformation is induced during the pregnancy, and if indused at an early stage, the presence or lack of p-glycoprotein in the fetus would be of no importance to the sensitivity. We would therefore agree with the MSCA submitting this proposal that these malformations may also be relevant to humans, and that the classification Repr. Carc. Cat.3, R63 is justified.

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		relevant to humans. Hence, there is currently no evidence for the existence of mutations in the human population that result in a loss of func tion analogous to that seen in the CF-1 mice. Furthermore, brain pgp expression starts early in human development, having been detected in human foetal brain micro vessels as early as week eight of pregnancy, on contrary to rats. Because of this early pgp expression in human foetal brain and the presence of pgp in placental mother/fetus barrier, we propose to remove the classification Repr. Carc. Cat.3; R63. Proposed labeling : T+, R26/28, R48/23/25; N, R50/53		

Carcinogenicity

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/05	Hungary / Zsuzsanna Kiss / National Institute of Chemical Safety	On the basis of the detailed information appended, we agree that abamectin is unlikely to pose a carcinogenic hazard.	Thank you for your support	The support is noted.
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 31 We support not to classify abamectin for carcinogenic hazard.	Thank you for your support	The support is noted.

Mutagenicity

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 30 We support not to classify abamectin for mutagenic hazard.	Thank you for your support	The support is noted.

Toxicity to reproduction

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/05	Hungary / Zsuzsanna Kiss / National Institute of Chemical Safety	We agree with the proposed classification: Repr. Cat. 3; R63	Thank you for your support	The support is noted.
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 32ff We support not to classify abamectin for toxic effects on fertility or effects during/on lactation. Nevertheless, it would have been helpful if the actual numbers of the fertility data had been included in the annex VI report itself (we noted them in the revised addendum to the draft assessment report). It would have been helpful if the draft assessment report and the addenda had been included into the dossier (now they were only available from the people involved in the pesticide evaluation).	Thank you for your support. We have included the table with the results of the final evaluation of the multigeneration reproductive toxicity study in the background document.	The support is noted.

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		We support to classify abamectin for toxic effects on development (R63, H361d). In rabbits of the high dose level of 2 mg/kg bw/d, 5 foetuses (3 litters) showed clubbed fore-foot. This finding was also observed in 1 foetus in control animals. Foetuses in 1 other litter in the high dose group showed cleft palate and omphaloceles (2 foetuses, each). Significant lower doe bodyweights and lower feed and water intakes were noted in the highest dose group. Therefore, the possibility that the developmental effects may have been due to unspecific influences such as generalised maternal toxicity can not be excluded. One rat foetus in the high dose group showed also cleft palate (one litter in historical control showed cleft palate; therefore, this finding in rats is considered of limited relevance). On the one hand, only one type of malformations occurred in one species in animals of the highest dose level. On the other hand, it might be discussed whether the incidences were high enough and the finding severe enough to consider them as "clear evidence".	Thank you for your support. As it is described in the C&L proposal the increased in incidence of clubbed fore-foot in rabbits is small but considered treatment-related. In addition, small increases in incidences of other malformations were observed (cleft palate, omphaloceles) in rats and rabbits. Based on the increase (but not clear increase) in malformations it is proposed to classify abamectin with Repr. Cat. 3; R63.	The support is noted.
2009/10/08	Denmark / Louise Grave-Larsen / MSCA	P. 36 the conclusion on developmental toxicity: Denmark supports the classification of Abamectin with Repr. Cat. 2, R61"may	In the developmental toxicity studies in rats and rabbits no treatment-related effects on brain development were observed. As it is described in the C&L proposal the increased in incidence of	Although malformations were noted in two species, we do not think that the evidence suffices for a Repr. Cat 2 classification.

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		cause harm to the unborn child". Justification: there is effects in two species both rat and rabbit. Even tough there is a time difference between human and rat concerning the P-glycoprotein expression in the blood/brain barrier, there is no data supporting that the higher concentration in rat brain during lactation is the sole cause of mortality. In addition, the human embryo will also be vulnerable until the BB barrier is established, and therefore a small window of opportunity can arise in the very early stages of pregnancy during the development of the neural tube. Denmark is therefore of the opinion that the data is inadequate to rule out human relevance.	clubbed fore-foot in rabbits is small but considered treatment-related. In addition, small increases in incidences of other malformations were observed (cleft palate, omphaloceles) in rats and rabbits. Based on the increase (but not clear increase) in malformations it is proposed to classify abamectin with Repr. Cat. 3; R63. We are of the opinion that the effects are not strong enough to justify a classification with Repr. Cat. 2 R61	
2009/10/16	Sweden / Swedish Chemicals Agency	The increase in malformations (clubbed fore-foot) in rabbits at the highest dose 2 mg/kg/day is above the concurrent and historic controls and therefore treatment related. The small reduction in the maternal body weight gain is unlikely the cause of this increased incidence in malformation. We agree to the conclusion in the proposal "As the time of development of this effect [clubbed fore-feet] is unknown; it is unknown whether the differences in p-glycoprotein development between rabbits and humans are also important for this effect. Therefore, it is assumed that	Thank you for your support	The support is noted.

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		this effect is also relevant to humans. "Therefore, the proposed classification of abamectin for harm to the unborn child as Repr. Cat. 3; R63 is justified according to Directive 67/548/EEC and as Repr. Cat. 2; H361d according to Regulation (EC) 1272/2008."		

Respiratory sensitisation

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 25 We support not to classify abamectin for respiratory sensitising hazard.	Thank you for your support	The support is noted.

Other hazards and endpoints

Date	Country / Person/Organisation/ MSCA	Comment	Response	Rapporteur's comment
2009/10/06	Germany / Bernd Niederstraßer / MSCA	The German CA is of the following opinion: Page 26ff We support to classify abamectin for specific organ toxicity-repeated exposure (R48/23/25, H372 [STOT-RE cat. 1]). Category 1 is justified because the substance causes neurotoxicity in rats and dogs at doses below 10 mg/kg bw/d (guidance value) at oral exposure.	Thank you for your support	The support is noted.

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		Furthermore, the results from an inhalation study in rats show neurotoxic effects at concentrations of 2.69 µg/L (guidance value: 60 µg/L in 30-d study). We support to classify abamectin for acute toxicity (R26-R28, H300-H330). The oral LD_{50} value in rats is 8.7 mg/kg bw in males and 12.8 mg/kg bw in females and justifies the classification with category 2 (guidance value in Reg. (EC) No. 1272/2008: 5 < 50 mg/kg bw). The $LC_{50} \leq LD_{50}$ values in rats in two different studies are < 0.21 mg/L and 0.034 < LC_{50} < 0.051 mg/L (guidance value in Reg. (EC) No. 1272/2008 for category 1: 0.5 mg/L).	Thank you for your support	The support is noted.
2009/10/15	United Kingdom / Audrey Pearson / MSCA	Environmental classification endpoint: Environmental Fate: • In our view, the role of photolysis (both in degradation and when considering aquatic toxicity results) needs further consideration. • It would be helpful to add further details for the photolysis studies (e.g. whether the light source was artificial or natural; test duration, temperature, water depth, etc) as these are important to enable interpretation of the results in the context of the European environment for classification. At the moment, the quoted DT_{50} is representative of summer at 40°N (Southern Europe) under clear skies.	We have added more detailed information on the photodegradation of abamectin and its degradation products. And discussed the relevance of the available information on photodegradation for classification of abamectin. In our view the photodegradation can not easily be used for classification purposes. In practice it will not be possible to easily demonstrate that photodegradation in water is significant in the environment. One of the reasons is that in most natural	Agree with MS reply, subject to some further discussion of potential toxicity of degradation products

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		However, further DT_{50} data are available for representative winter and alternative EU conditions. These should also be included to allow consideration of photodegradation across the EU. • Given the relatively short half-lives, is there any information on degradation products? • While the results of the environmental simulation studies are presented, we think some further interpretation of the results is required, given the photodegradation potential. In addition, we feel it is more appropriate to present results such as DT_{50}s, as a range rather than averages. • The evidence for Abamectin's overall fate should be summarised in Section 4.1.3 and a clear conclusion given in relation to the classification criteria (i.e. why does the substance not meet the criteria for rapidly degradable?) in Section 7.6. Environmental Hazard: • It would be helpful to provide more detail and a more robust evaluation/consideration of the most critical/relevant studies, for example to state why a non-standard species EC_{50} is relevant for the purpose of classification and labelling, and to indicate that the studies meet relevant validation criteria. • Many of the ecotoxicity studies were carried out in the light, some under static	water bodies, the rate of photoreaction is affected by dissolved and suspended matter. Since the concentration of the substance under consideration is normally low compared to the concentration of e.g. dissolved humic acids, the natural constituents absorb by far the larger portion of the sunlight penetrating the water bodies. For this reason the DT_{50} values for the whole water/sediment system is considered most appropriate for the classification and labelling, based on which abamectin does not meet the criteria for readily biodegradable of both Directive 67/548/EEC and Regulation (EC) 1272/2008. We have added the information that all studies were conducted following internationally accepted methods. Water quality parameters of all test media were within accepted range and no control mortality was observed. We have added the following text: The difference between the static LC_{50} of	

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		exposure, and are reported as nominal concentrations. Given that Abamectin is susceptible to photolysis and adsorption, losses could be a possibility. This means it is currently difficult to assess how robust the nominal results are for the purposes of classification. While the classification is based on measured concentration data for an invertebrate, it is still relevant to consider this issue for other species since their $L(E)C_{50}$ values could be significantly lower if losses occurred (i.e. the key Mysid data might not be so much of an outlier as they appear at first sight). • It is normal to present toxicity data for algae as part of an environmental classification proposal, so it is unclear why they are not presented in the report when data are available in the DAR. We accept that they do not affect the classification, but think a more 'rounded' view of the dataset and the reasons why the classification was reached would be presented if this information was included. Classification Conclusion: • This section would benefit from clarifying which data are used as the basis of the classification and why.	0.21 µg/L for the saltwater species Mysidopsis bahia and the results of the flow-trough experiments (LC_{50} 0.020 and 0.022 µg/L) may be explained by the fact that the exposure concentration under flow through conditions remain constant whereas under static conditions losses could have occurred due adsorption and photodegradation. It should be noted that the LC_{50} obtained under static conditions is in the same order of magnitude as the LC_{50}s obtained for the fresh water invertebrates. The LC_{50} obtained under flow through conditions is considered most appropriate for the classification and labelling of abamectin. For the sake of completeness we have added the following text: Studies with the parent compound were performed at concentrations far above the water solubility and were therefore not accepted. The data does however show that algae are not more sensitive than crustaceans or fish. Further explanation is given which values are used for the classification. Further justification is given in the relevant chapters 4 and 7.	
2009/10/15	United Kingdom / Audrey Pearson /	**Section 4 Environmental Fate** • Section 4.2.2 (Volatilisation) - It	Thank you for your support.	Agree with MS reply

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	MSCA	would be helpful to provide a Henry's Law Constant and overall statement/ conclusion on what the data mean, i.e. whether or not the substance is likely to partition to air in aquatic tests. • Section 4.3.1.1 (Bioaccumulation estimation) - This section presents a Kow value but we think it should make some further comment about its relevance for bioaccumulation. • Section 4.3.1.2 (Measured bioaccumulation data) - It would be useful to include further study details (available in the DAR) such as fish species, uptake and depuration duration, why only one test concentration, etc. The viscera BCF of 110 l/kg should also be presented. • Section 4.3.3 (Summary and discussion of bioaccumulation) - When presenting the conclusions on bioaccumulation potential it would be useful to compare actual data against the bioaccumulation criteria (e.g. BCF<500) – hence making it clear the basis on which the conclusion was reached. **Section 7 (Environmental Hazard Assessment)** • Are any ecotoxicity studies using degradants available? • For those not familiar with Latin names it is worth including common	We would like to thank the UK for their detailed editorial comments. We agree with most of them and revised the background document accordingly. Regarding your remark on the justification for the use of ecotoxicity data using a 'non-standard' species. According to the CLP Regulation, both freshwater and marine species toxicity data are considered suitable for use in classification provided the test method equivalent to standardised test methods. This justification is added.	

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		names of species in tables 7.1-1, 7.1-2, 7.1-4. • Section 7.1.1.1 (Fish) - We do not feel the Peither (2003) study based on modified exposure should be included for the purpose of classification given the diminishing exposure and the fact that more representative data are available. • Section 7.1.1.5 (Other aquatic organisms) - Rather than include marine fish and invertebrates under this heading, it may be more appropriate to include them in sections 7.1.1.1 and 7.1.1.2 respectively (either under a separate section for marine/saltwater species or combined in a table for fish and a table for invertebrates). This would allow a comparison to be made of all species representative of a specific trophic level (and we think this is important because the key data for classification are presented in this section at the moment). • Table 7.1-4 should explain why two values are presented for the Suprenant (1988) study (i.e. the basis for the $L(E)C_{50}$ value of 0.020 µg/l is ≤ 1 day old organisms). **Section 7.6 (Conclusion on the environmental classification and labelling)**		

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		• The statement 'The available EC_{50}s values ranged from 0.0035 µg/l to 6.1 µg/l' should read as 'NOECs' not EC_{50}s (and it should be noted that this range included values for mortality and reproduction). However, given that the classification is based on an acute LC_{50} study, is there any need for this comment?		
2009/10/16	Sweden / Swedish Chemicals Agency	Severe neurological effects occur after administration of low doses of the substance. Therefore, the classification proposed for repeated dose toxicity is supported.	Thank you for your support	The support is noted.