European Chemicals Agency

COMMITTEE FOR RISK ASSESSMENT

BACKGROUND DOCUMENT TO THE OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING OF

ABAMECTIN
CAS number: 71751-41-2

EC number: n.a.

AND

AVERMECTIN B_{1A}
CAS number: 65195-55-3
EC number: 265-610-3

FINAL
17 March, 2010

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1. TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Names:	***abamectin* (combination of avermectin B_{1a} and avermectin B_{1b})**	***avermectin B_{1a}* (purity more than 80 %)**
EC Number:	***n.a.***	***265-610-3***
CAS Number:	***71751-41-2***	***65195-55-3***

Registration number (s): CIPAC 495 (collaborative international pesticides analytical council code number)

Purity[1]: Min. 90 % w/w abamectin (sum of avermectin B_{1a} and avermectin B_{1b})
Min. 83 % w/w avermectin B_{1a}
Max. 8 % w/w avermectin B_{1b}

Impurities: Based on the available environmental and (eco)toxicological information, there are no relevant impurities

Remark:

The present proposal for harmonized Classification and Labelling applies to the technical active substance abamectin as proposed for inclusion in Annex I of Council Directive 91/414/EEC and Annex I of Directive 98/8/EC and its main component avermectin B1a.

Confidential information on the content and identity of isomers, impurities and additives is available in Volume 4, Annex C of the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands).

Proposed classification based on Directive 67/548/EEC:

Phys/Chem hazards: -

Health hazards:

Repr. Cat.3; R63

T+; R26/28

T ; R48/23/25

Environment: **N; R50/53**

Proposed classification based on Regulation EC 1272/2008:

[1] Applies to abamectin only.

Signal word: **Danger**

Phys/Chem hazards: **-**

Health hazards:

Repr. 2 H361d

Acute Tox. 2 H300

Acute Tox. 1 H330

STOT-RE 1 H372 (Causes damage to the nervous system through prolonged or repeated exposure)

Environment:

Aquatic Acute 1 H400

Aquatic Chronic 1 H410

Proposed labelling:

Directive 67/548/EEC:

Symbol : **T+, N**

Risk phrases : **R26/28-R48/23/25-R63-R50/53**

Safety phrases : **S28-S36/37-S45-S60-S61**

Regulation (EC) 1272/2008:

Signal word: **Danger**

Symbol: **GHS06, GHS08, GHS09**

Hazard statement codes: **H300, H330, H361d, H372, H400, H410**

As precautionary statements are not included in Annex VI of Regulation EC 1272/2008, no proposal is made.

Proposed specific concentration limits (if any):

Specific concentration limits:

Cn ≥ 5% T ; R48/23 ; STOT-RE 1; H372 Causes damage to the nervous system through prolonged or repeated exposure

0.5% ≤ Cn <5% Xn; R48/20; STOT-RE 2; H373 May cause damage to the nervous system through prolonged or repeated exposure

Classification of the preparation/mixture		
N; R50-53 H400, H410	N; R51-53 H411	R52-53 H412
Cn ≥ 0.0025%	0.00025% ≤ Cn <0.0025%	0.000025% ≤ Cn <0.00025%

where Cn is the concentration of abamectin/avermectin B_{1a} in the preparation/mixture.

M-factor for 67/548 EEC and EC 1272/2008:

The M-factor is 10,000. This value is based on two LC_{50} values of 0.020 µg/l and 0.022 µg/l obtained for the marine crustacean *Mysidopsis bahia* in a 96-h flow-through study.

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Substance Names:	***abamectin* (combination of avermectin B_{1a} and avermectin B_{1b})**	***avermectin B_{1a}* (purity more than 80 %)**
EC Number:	***n. a.***	***265-610-3***
CAS Number:	***71751-41-2***	***65195-55-3***

Abamectin is the ISO common name for a mixture of ≥80 % avermectin B_{1a} and ≤20 % avermectin B_{1b}. The use of the word "mixture" in the ISO description is not in line with REACH and CLP terminology. Following the terminology of REACH and CLP Regulations, abamectin is a substance containing ≥80 % avermectin B_{1a} and ≤20 % avermectin B_{1b}.The material considered in this report fulfils the ISO definition.

According to the REACH guidance document on substance identification the substance is a mono-constituent substance with avermectin B_{1a} (CAS Number 65195-55-3) as its main constituent (purity > 83%) and with avermectin B_{1b} as an impurity. However, part 1.1.1.4 of Annex VI of Regulation (EC) 1272/2008 (CLP Regulation) states that whenever possible plant protection products and biocides are designated by their ISO names. As abamectin is used as both a plant protection product and as a biocide in this proposal preference is given to the use of the ISO name abamectin as the International Chemical Identifier for inclusion in Annex VI of the CLP Regulation.

1.2 Composition of the substance

The present proposal for harmonized Classification and Labelling applies to the technical active substance abamectin and its main component avermectin B_{1a} as proposed for inclusion in Annex I of Council Directive 91/414/EEC and Annex I of Directive 98/8/EC.

Confidential information on the content and identity of isomers, impurities and additives is available in Volume 4, Annex C of the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands).

Purity and impurities[2]

[2] Applies to abamectin only.

Purity: Min. 90 % w/w abamectin (sum of avermectin B_{1a} and avermectin B_{1b})
Min. 83 % w/w avermectin B_{1a}
Max. 8 % w/w avermectin B_{1b}

Main constituent

Chemical Name: avermectin B_{1a}

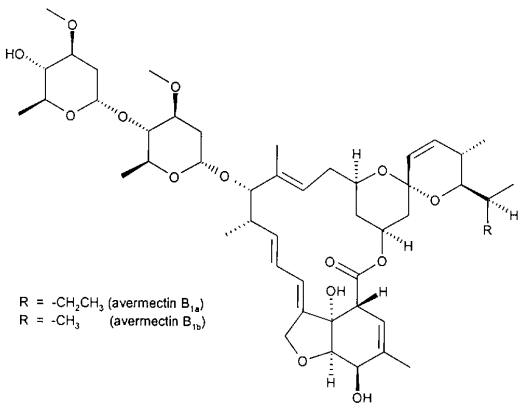
EC Number: 265-610-3

CAS Number: 65195-55-3

IUPAC Name: (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-6'-[(*S*)-*sec*-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-O-methyl-*α*-L-*arabino*-hexopyranosyl)-3-*O*-methyl-*α*-L-*arabino*-hexopyranoside

Molecular Formula: $C_{48}H_{72}O_{14}$

Structural Formula:



Molecular Weight: 873.1

Typical concentration (% w/w): ≥ 83%

Concentration range (% w/w): confidential

Impurity

Chemical Name:

avermectin B_{1b}:

EC Number: 265-611-9

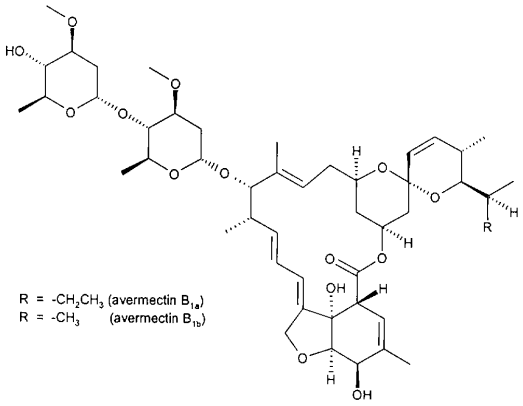
CAS Number: 65195-56-4

IUPAC Name: (10*E*,14*E*,16*E*,22*Z*)-(1*R*,4*S*,5'*S*,6*S*,6'*R*,8*R*,12*S*,13*S*,20*R*,21*R*,24*S*)-21,24-dihydroxy-6'-isopropyl-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene-6-spiro-2'-

(5',6'-dihydro-2'*H*-pyran)-12-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-*α*-L-*arabino*-hexopyranosyl)-3-*O*-methyl-*α*-L-*arabino*-hexopyranoside

Molecular Formula: $C_{47}H_{70}O_{14}$

Structural Formula:



Molecular Weight: 859.1

Typical concentration (% w/w): ≤ 8%

Concentration range (% w/w): confidential

Other impurities:

The natural fermentation process for the production of abamectin produces several impurities, which are structurally similar to avermectin B_{1a} and avermectin B_{1b}. Because of their low concentration level and their expected similar (eco)toxicity to avermectin B_{1a} and avermectin B_{1b}, these impurities are considered not (eco)toxicologically relevant in the material (see DAR October 2005 + addendum February 2008, RMS The Netherlands and the CAR; July 2008; RMS The Netherlands).

Test material:

The active substance abamectin, produced in a natural fermentation process, contains both avermectin B_{1a} and avermectin B_{1b}. All studies, unless otherwise stated, were carried out using abamectin which varied in purity between 88.3 and 96.7% (sum of avermectin B_{1a} and avermectin B_{1b}). Where information on the ratio between avermectin B_{1a} and avermectin B_{1b} was available for batches used in the toxicological studies, these were above 80% for avermectin B_{1a} and below 20% for avermectin B_{1b}. Studies not carried out using abamectin were mostly conducted with the major component avermectin B_{1a}.

The variation in purity and ratio is not expected to substantially affect the fate, toxicity and the classification and labelling.

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	Powder at 25 ºC (96.7% w/w)
VII, 7.2	Melting/freezing point	3.2	161.8 °C – 169.4 °C (96.7% w/w) with thermal decomposition during melting (at 162 °C)
VII, 7.3	Boiling point	3.3	Not determined, due to thermal decomposition during melting of abamectin
VII, 7.4	Relative density	3.4 density	1.18 at 22 °C (96.7% w/w)
VII, 7.5	Vapour pressure	3.6	< 3.7×10^{-6} Pa at 25 ºC (96.7% w/w) using the gas saturation method
VII, 7.6	Surface tension	3.10	52.4 mN/m at 90% of the saturation concentration at 20 °C (purity 96.7% w/w)
VII, 7.7	Water solubility	3.8	Water solubility at 25 ºC (purity not stated) using the shake flask method pH 7.57: 1.21 mg/L (in water)
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log Kow = 4.4 at pH 7.2 at 20 ºC (water), (purity 96.7% w/w) using shake flask method
VII, 7.9	Flash point	3.11	No data
VII, 7.10	Flammability	3.13	Abamectin is considered to be not highly flammable
VII, 7.11	Explosive properties	3.14	No explosive properties
VII, 7.12	Self-ignition temperature		No self-ignition was observed before the melting point
VII, 7.13	Oxidising properties	3.15	No oxidising properties
VII, 7.14	Granulometry	3.5	No data
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	No data
XI, 7.16	Dissociation constant	3.21	No dissociation in the pH-range from 1 to 12
XI, 7.17	Viscosity	3.22	Not data, Abamectin is a solid, not a liquid
	Auto flammability	3.12	No data
	Reactivity towards container material	3.18	Abamectin is packed in a conical bucket inside another conical bucket (inside the buckets are two polyethylene bags which contain the material). The registrant indicates that there is no record of any reaction to the container material

	Thermal stability	3.19	Thermal decomposition during melting (above 162 °C, 96.7% w/w) Identification of breakdown products was not performed. The registrant indicates that combustion products are likely to be oxides of carbon and water. The registrant indicates that dangerous products are unlikely to be formed.

The above data are obtained from the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands) and the Competent Authority Report (CAR; July 2008; RMS The Netherlands) on the inclusion of abamectin in Annex I to Directive 98/8/EC concerning the placing of biocidal products on the market.

2 MANUFACTURE AND USES

Abamectin is used as an insecticide and acaricide for the control of motile stages of mites, leaf mines, suckers, Colorado beetles, etc. on ornamentals, cotton, citrus fruit, pome fruit, nut crops, vegetables, potatoes and other crops. Also used for the control of fire ants.

The assessment of the biocidal activity of the active substance demonstrates that it has a sufficient level of efficacy against some of the target organisms (pharao ants and cockroaches)

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Current classification: None

3.2 Self classification(s)

The registrant has proposed the following classification and labelling of the active substance abamectin.

Proposal of the registrant according to Directive 67/548/EEC for abamectin:

Hazard symbol:	T+ N	Very toxic Dangerous for the environment
Risk phrases	R26 R28 R50/53	Very toxic by inhalation Very toxic if swallowed Very toxic to aquatic organisms, may cause long-

		term adverse effects in the aquatic environment
Safety phrases	S28	After contact with skin, wash immediately with plenty of ... (to be specified by the manufacturer)
	S36/37	Wear suitable protective clothing and gloves
	S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/safety data sheet

Proposal of the registrant according to Regulation (EC) 1272/2008: No proposal

4 ENVIRONMENTAL FATE PROPERTIES

The environmental fate properties assessment for abamectin is based on the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands) and the Competent Authority Report (CAR; July 2008; RMS The Netherlands) on the inclusion of abamectin in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

All tables in the present assessment are copied from the DAR or CAR. The tables are renumbered in accordance with the paragraph numbers in Chapter 4.

4.1 Degradation

4.1.1 Stability

Hydrolysis

Both ^{14}C- and ^{3}H-avermectin B_{1a} are hydrolytically stable at environmentally relevant pH (4 - 7) and temperature (25 °C). Under basic conditions (pH 9), $DT_{50,hydrolysis}$ of avermectin B_{1a} was 213, 9.9 and 4.9 days at 25, 50 and 60 °C, respectively, and the calculated $DT_{50,hydrolysis}$ at 20 °C is 380 days.

Table 4.1-1 Hydrolysis of abamectin

Guideline/ Test method	Substance	pH	Temperature [℃]	Initial test substance concentration, C_0 [mg/L]	Reaction rate constant, K_h [1/d][a]	Half-life, $DT_{50,hydro}$ [d]	Reference
OECD 111; EPA N 161-1; BBA 55, I and II	^{14}C-avermectin B_{1a}	4	50	0.11 (with 20% acetonitrile)		no hydrolysis	Ellgehausen, 2001
		5	50	0.11 (with 20% acetonitrile)		no hydrolysis	
		7	50	0.11 (with 20% acetonitrile)		no hydrolysis	
		9	25	0.11 (with 20% acetonitrile)	3.25×10^{-3}	213	
		9	50	0.11 (with 20% acetonitrile)	0.07	9.9	
		9	60	0.11 (with 20% acetonitrile)	0.14	4.9	

a: calculated as $\ln 2/DT_{50}$

Photolysis in water

The data on aqueous photodegradation of avermectin B_{1a} are summarised in Table 4.1-2.

Table 4.1-2 Photolysis of avermectin B_{1a} in water

Guideline/ Test method	Substance	Initial test substance concentration, C_0 [µg/L]	Total recovery of test substance [% of added radioactivity]	Photolysis rate constant, K^c_p [1/d] [a]	Reaction quantum yield [Φ^cE]	Half-life, $DT_{50,photo}$ [d]	Reference
EPA 161-2	^{14}C-avermectin B_{1a}	100 (in 1% acetonitrile)	91.8	0.35		2	Adam, 2001b
EPA 188	^{3}H-avermectin B_{1a}	3 (in 1% acetonitrile)	93.3	0.53	0.0287-0.0347	1.3	Halley et al., 1991

a: calculated as $\ln 2/DT_{50}$

In the first study (Adam, 2001b), samples were irradiated in a Suntest exposure unit with a Xenon lamp with UV-filter (λ > 290 nm), with a light/dark schedule of 12 hours light and 12 hours dark. The incubation was carried out for 37.5 days. The DT50, photolysis for avermectin B_{1a} of 2 days obtained is equivalent to 1.5 sunlight days at 30 - 50 °N.. Up to 30 unknown fractions were detected, individual compounds accounted for at most 6.6 % of the added radioactivity. [8,9-Z]-

avermectin B_{1a} was formed up to 8.2 % with estimated DT50,photolysis of 7.6 days (5.8 sunlight days at 30 - 50 °N).

In a second study by Halley et al., (1991) samples were incubated for 6 days under natural daylight conditions receiving about 8 hours of sunlight/day. Temperature in the samples, measured around noon each day, was between 22.0 and 32.0 °C, average 26.6 °C. Quantum yield was determined to be 0.0347, 0.0316 and 0.0287 at 40 °N in summer, fall and winter, respectively. Corresponding $DT_{50,photolysis}$ values for summer, fall and winter at a flat water surface under clear skies were 1.32 days, 2.88 days and 5.08 days, respectively.

In a third study by Ku & Jacob (1983) performed at natural sunlight, the temperature and light intensity were not given and therefore the obtained DT_{50}'s were not considered acceptable. This study does however shows that the same degradation product [8,9-Z]-avermectin B_{1a} was formed as observed in the first study (around 12%) and > 10 % formation of unknown degradation products. In an addendum it is stated that the non-polar and moderately polar fractions are transient metabolites that are further degraded into the polar fraction. This fraction contains multiple peaks and, according to an internal memo, is > 160 times less toxic to *Daphnia magna* than avermectin B_{1a}.

Photolysis in soil

The data on photodegradation of avermectin B_{1a} in soil are summarised in Table 4.1-3.

Table 4.1-3 Photolysis of avermectin B_{1a} in soil

Guideline/ Test method	Substance	Initial tests substance application rate, C_0 [kg/ha]	Total recovery of test substance [% of added radioactivity]	Photolysis rate constant, K^c_p [1/d][a]	Reaction quantum yield [$\Phi^c E$]	Half-life, $DT_{50,photo}$ [d]	Reference
EPA 161-3	^{14}C-avermectin B_{1a}	0.09	100	0.05		13	Phaff, 2001

a: calculated by RMS as $ln2/DT_{50}$

The $DT_{50,photolysis}$ of 13 days is equivalent to 22 days at 30 – 50 °N. Mineralization and bound residues were 7.6 and 25.9 %, respectively, after 28 days.

Photo-oxidative degradation in air

The atmospheric half-life time of abamectin is estimated according to Atkinson as < 1 hour (Stamm, 1998).

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

No data available.

4.1.2.2 Screening test

Readily biodegradability

Abamectin at concentration (100 mg/L) in the study of Dietschy (1999) was far above the solubility in water of 1.21 mg/L but within the concentration range recommended by the test guideline. In the absence of other data, abamectin is considered as *not readily biodegradable*.

Table 4.1-4 Ready biodegradability of abamectin

Guideline/ Test method	Test type	Test parameter	Inoculum			Additional substrate	Test substance concentration	Degradation		Remarks	Reference
			Type	Concen-tration	Adaptation			Incubation period [d]	Degree [%]		
OECD 301F	manometric respirometry	oxygen demand	STP	26 mg/L		synthetic	100 mg/L	28	3		Dietschy (1999)

4.1.2.3 Simulation tests

Biodegradation in water/sediment systems

Aerobic water/sediment system

In the study of Buckel (2002), a sandy loam system (River Rhine) and a silty clay loam system (pond) were treated with 14C-avermectin B1a and incubated under aerobic or anaerobic conditions at 20 °C in the dark. The results of the aerobic incubation are summarised in Table 4.1-5.

Table 4.1-5 Degradation of abamectin in *aerobic* water/sediment systems

Guideline/ Test method	Substance	System name	Sediment type	Condition	T [°C]	pH_{water}	OM [%]	Duration [d]	DT_{50} water[1] [d]	DT_{50} sediment [d]	DT_{50} system [d]	Reference
OECD draft 2000; BBA IV, 5-1	^{14}C-avermectin B_{1a}	River Rhine	sandy loam	aerobic	20	7.9-8.4	2.5	100	1.8	87	87	Buckel, 2002
OECD draft 2000; BBA IV, 5-1	^{14}C-avermectin B_{1a}	Rothenfluh pond	silty clay loam	aerobic	20	7.7-8.4	7.7	100	2.9	111	91	Buckel, 2002

1: $DT_{50,water}$ determined by sorption, value represents dissipation

The decline of concentrations in the water phase was mainly determined by a rapid initial sorption, and the DT50,water thus represents dissipation rather than degradation.

The maximum level of avermectin B1a found in sediment was 78.1% of added radioactivity (pond) and 82.8% of added radioactivity (river) after 14 days. At the end of the study after 100 days, levels of avermectin B1a had declined to 44.3 and 45.3% of added radioactivity for river and pond, respectively.

Bound residues increased to 20.4% of added radioactivity (river) and 23.2% of added radioactivity (pond) at the end of the study after 100 days, mineralisation was low with a maximum of 3.0 and 3.2% of added radioactivity after 100 days in the river and pond system, respectively.

Anaerobic water/sediment system

In the *anaerobic* systems, dissipation from the water phase was fast (DT50,water of 5.6-7.2 days, see table 4.1-6), but degradation in the total system was much slower with < 50% degradation at the end of the study after 100 days (see Table 4.1-6). DT50,sediment-values could not be estimated because there were too few data points with decline.

Table 4.1-6 Degradation of abamectin in *anaerobic* water/sediment systems

Guideline/ Test method	Substance	System name	Sediment type	Condition	T [°C]	pH_{water}	OM [%]	Duration [d]	DT_{50} water[1] [d]	DT_{50} sediment [d]	DT_{50} system[2] [d]	Reference

Guideline/ Test method	Substance	System name	Sediment type	Condition	T [°C]	pH_{water}	OM [%]	Duration [d]	DT_{50} water[1] [d]	DT_{50} sediment [d]	DT_{50} system[2] [d]	Reference
OECD draft 2000; BBA IV, 5-1	^{14}C-avermectin B_{1a}	River Rhine	sandy loam	anaerobic	20	8.4-9.2	2.5	100	7.2		230	Buckel, 2002
OECD draft 2000; BBA IV, 5-1	^{14}C-avermectin B_{1a}	Rothenfluh pond	silty clay loam	anaerobic	20	7.8-9.8	7.7	100	5.6		312	Buckel, 2002

1: $DT_{50,water}$ determined by sorption, value represents dissipation
2: extrapolated value

Biodegradation in soil

Aerobic biodegradation

The rate of degradation of avermectin B_{1a} under aerobic conditions was assessed in four laboratory experiments in eight different soil types. The experiments are summarised in Table 4.1-7. Results from the study of Ku and Jacob (1983a) indicate that there is no clear relation between dose and degradation rate. This study was performed at ambient temperature, which is supposed to be 20 °C. Moisture content may influence the degradation rate: at 30 °C, the DT_{50} under dry conditions (pF 4) is 24.4 days, which is higher than the value found at field capacity (16.6 days).

Where multiple experiments were performed with a single soil type under the same conditions (temperature, moisture), the geometric mean of the DT_{50}-values is calculated.

Table 4.1-7 Overview of DT_{50}-values from aerobic laboratory degradation studies with avermectin B_{1a}

Guideline/ Test method	Label	Soil type	Dose [mg/kg]	T [°C]	OM [%]	pH	pF	DT_{50} [d]	DT_{50}, 20 °C [d]	Reference
BBA IV, 4-1; draft OECD	^{14}C	loam	0.22	20	3.2	7.3	2	18.8	18.8	Nicollier, 2001
BBA IV, 4-1; draft OECD	^{14}C	silt loam	0.1	20	4	7.2	2.5	23.3	23.3	Adam, 2001a
	^{14}C	silt loam	0.1	10	4	7.2	2.5	50.6[1]		
	^{14}C	silt loam	0.1	30	4	7.2	2.5	16.6		
	^{14}C	silt loam	0.1	30	4	7.2	4	24.4		
BBA IV, 4-1; draft OECD	^{14}C	loamy sand	0.125	20	2.4	7.41	2.5	23.6	23.6	Phaff, 2003
	^{14}C	sandy clay loam	0.125	20	4.3	5.81	2.5	11.2	11.2	
	^{14}C	silty clay loam	0.125	20	2.4	7.92	3.5	49.6	49.6	
not specified	^{3}H	sandy loam	0.1	ambient	1.1	6.8	2.5	26.9	geometric	Ku & Jacob, 1983a
	^{3}H	sandy loam	1	ambient	1.1	6.8	2.5	22.3	mean for	
	^{3}H	sandy loam	50	ambient	1.1	6.8	2.5	42.6	sandy loam:	
	^{14}C	sandy loam	1	ambient	1.1	6.8	2.5	15.1	28.3	
	^{14}C	sandy loam	1	ambient	1.1	6.8	2.5	47.0		
	^{3}H	sand	1	ambient	0.6	8	2.5	65.7	65.7	
	^{3}H	clay	0.1	ambient	1.3	6.8	2.5	34.9	geometric for	
	^{3}H	clay	1	ambient	1.3	6.8	2.5	44.9	clay: 39.6	

1: actual temperature 8.6 °C, value at 10 °C estima ted using the Arrhenius equation

The overall geometric mean DT50 of avermectin B1a at 20 °C is 28.4 days (range 11.2 – 65.7 days; n = 8; r2 0.9471 - 0.9970).

The highest formation of bound residues was 39.1% of added radioactivity after incubation for 91 days at 20 °C and further increased to 44.1% of added radioactivity after 196 days (Phaff, 2003). Highest mineralisation accounted for 12.4% of added radioactivity after 91 days (Phaff, 2003) and reached 27.6% of added radioactivity in another study at the end of a 365-days incubation period (Nicollier, 2001).

Based on degradation rates at 8.6, 20 and 30 °C (Adam, 2001a), the DT50 of avermectin B1a at 10 °C is estimated as 50.6 days.

4.1.3 Summary and discussion of persistence

Biodegradation in water

Abamectin was found to be not readily biodegradable in a ready biodegradability study.

In natural aerobic water/sediments systems, the dissipation of abamectin from the water phase was dominated by sorption with a $DT_{50,water}$ of 2.4 days. The average $DT_{50,system}$ was 89 days whereas the $DT_{50,sediment}$ was 99 days.

In natural anaerobic water/sediment systems, dissipation of abamectin was fast with $DT_{50,water}$ of 6.4 days. In contrast, $DT_{50,sediment}$ could not be determined due to limited degradation. The $DT_{50,system}$ was on average 271 days.

Under both natural and artificial light condition the half-live of abamectin was between 1 and 2 days. The estimated half-live for one of the major photolytic product [8,9-Z]-avermectin B_{1a} was 7.6 days.

In the REACH guidance it is stated that 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 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 DT50 values for the whole water/sediment system is considered most appropriate for the classification and labeling, based on which abamectin does not meet the criteria for readily biodegradable of both Directive 67/548/EEC and Regulation (EC) 1272/2008.

Biodegradation in soil

The geometric mean DT50 of avermectin B1a in soil at 20 °C is 28.4 days. The highest formation of bound residues was 39.1% of added radioactivity after incubation for 91 days at 20 °C. Highest mineralisation accounted for 12.4% of added radioactivity after 91 days.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

Batch equilibrium experiments have been performed with avermectin B_{1a} in eight different soils. One of the soils was a sand with 0.1% OM (0.06% OC), which is considered not relevant for risk assessment. Accepted K_{OC}-values are summarised in Table 4.2-1. The average K_{OC} is 5638 L/kg (range 1495 – 7893; n = 7). Sorption of avermectin B_{1a} is related to OC-content, linear regression of K_F versus % OC gives a regression coefficient r^2 of 0.919. Abamectin can be considered as immobile in soil.

Table 4.2-1 Adsorption of avermectin B_{1a} onto soils

Guideline /	Adsorbed	K_a [1]	K_{aOC} [2]	K_d [3]	K_{dOC} [4]	K_a / K_d [5]	Degradation products	Remarks	Reference

							Name	[%] of a.s.		
^{14}C-avermectin B_{1a}										
OECD 106		87.2	5701				test substance was stable during		loamy sand	Morgenroth,
OECD 106		77.3	7893				mass balance experiment		loamy sand	2001
OECD 106		76.8	6004						sandy loam	
OECD 106		178	6875						loam	
OECD 106		334	6682						silt loam	
^{3}H-avermectin B_{1a}										
not spec.		18.2	1495				test substance was stable during		silt loam	Gruber &
		134	4814				mass balance experiment		clay loam	Wislocki, 1988
		average	5638							

1: K_a = Adsorption coefficient	4: K_{doc} = Desorption coefficient based on organic carbon content
2: K_{aoc} = Adsorption coefficient based on organic carbon content	5: K_a / K_d = Adsorption / Desorption distribution coefficient
3: K_d = Desorption coefficient	

4.2.2 Volatilisation

Abamectin has vapour pressure of $<3.7 \times 10^{-6}$ Pa, and a Henry's law constant of $\leq 2.7 \times 10^{-3}$ Pa·m^3·mol^{-1}, or in dimensionless form $\leq 1.13 \times 10^{-6}$. Based on this information it is considered that significant volatilisation of abamectin from soil or water is considered to be low.

4.2.3 Distribution modelling

Not relevant for this dossier

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Abamectin has a log Kow of 4.4 at pH 7.2 at 20 ºC.

4.3.1.2 Measured bioaccumulation data

Bluegill sunfish were exposed to ^{3}H-avermectin B_{1a} for 28 days to measure uptake of the compound and then placed in clean water for 14 days to determine elimination rate. Fish had mean weight 6.2 g and mean length 55 mm at test initiation. A flow-through system with continuous aeration, 70 L test solution per system, 110 fish per system were used. Actual water concentrations in the treated system were 0.099 ± 0.019 µg/L during the uptake period. Bioconcentration factors were determined by calculating the ratio of the total radioactive residues in fish (plateau values) and the average concentration of the test substance in the water.

Concentrations of ^{3}H-avermectin B_{1a} in fish increased until day 10. Plateau concentrations were 6.8 µg/kg wwt for whole fish, 3.0 µg/kg wwt for fillet and 11 µg/kg for viscera, resulting in bioconcentration factors of 69, 30 and 110 L/kg wwt, respectively. During the elimination phase of 14 days, ^{3}H-avermectin B_{1a} decreased to 0.32 µg/kg wwt in whole fish. Uptake rate constant was 11 L/kg wwt.d and elimination rate constant 0.21/d. Based on the fitted uptake and elimination rate constants, the BCF is 52 L/kg ww.

Table 4.3-1 Measurements of aquatic bioconcentration

Guideline / Test method	Exposure	Log P_{OW}	Initial concentr. [µg/L]	Steady-state BCF [L/kg ww]	Uptake rate constant [mL/g.d]	Depuration rate constant [1/d]	Depuration time (DT_{50}) [d]	Metabolites	Remarks	Reference
ASTM 1978	flow-through	4.4	0.1	69[1] 52[2]	11	0.21	3.3	N.D.[3]	whole fish; based on TRR	Forbis & Franklin, 1983

1: based on plateau TRR in whole fish and average TRR in water
2: estimated from uptake and elimination rate constants
3: ND, Not determined

4.3.2 Terrestrial bioaccumulation

4.3.3 Summary and discussion of bioaccumulation

Abamectin has a log Kow of 4.4. However, a BCF of 52 L/kg ww (based on the total radioactive residue) and 69 L/kg (based on whole fish) was obtained in a bioaccumulation study. Based on the results of the bioaccumulation study, abamectin does not significantly bioaccumulate and does not meet the criteria for classification based on bioaccumulation according to Directive 67/548/EEC or Regulation EC 1272/2008.

4.4 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazard assessment for abamectin is based on the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands), the EFSA conclusion (EFSA Scientific Report (2008) 147, 1-106) and the Competent Authority Report (CAR; January 2009; RMS The Netherlands) on the inclusion of abamectin in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

It should be noted that in the present human health hazard assessment most data on studies in CF-1 mice are excluded. Many studies with abamectin were performed with the CF-1 mouse, which is very sensitive to the observed developmental effects. Based on a recent extensive overview of the literature, it was however concluded that the CF-1 mouse is not relevant for human risk assessment (see 5.10.1) because some CF-1 mice lack the p-glycoprotein which has a function in restricting the brain penetration of avermectins including abamectin. Absence of p-glycoprotein is not known to occur in humans. The results of studies with the CF-1 mouse are therefore also not relevant for classification and labelling.

All tables in the present human health hazard assessment are copied from the DAR or CAR. The tables are renumbered in accordance with the paragraph numbers in chapter 5.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

5.1.1 Absorption

5.1.1.1 Oral Absorption

Avermectin B_{1a}, administered in sesame oil or in polyethylene glycol, is absorbed from the gastrointestinal tract of the rat and is distributed throughout all major tissues and organs sampled. Maximum concentrations in blood are achieved within 4-8 h after administration.

The comparison of urinary excretion after oral or i.v. administration indicates almost complete oral absorption, with a calculated bioavailability of 0.86.

5.1.1.2 Inhalation Absorption

For the inhalation route no data are available. Absorption through inhalation is assumed to be 100%.

5.1.1.3 Dermal Absorption

The extent of dermal penetration of avermectin B_{1a} is minimal in the rhesus monkey, amounting to less than 1% of the applied dose. The low dermal absorption of <1% was confirmed by a recent *in vitro* dermal absorption study with human skin (see revised addendum to pesticides Draft Assessment Report, February 2008).

5.1.2 Distribution

Avermectin B_{1a} and/or metabolites do not accumulate in liver, kidneys, muscle or fat on repeated administration of a low dose. Seven days after the last of 14 daily consecutive doses less than 1% of the total administered dose was present in tissues and organs. The highest residue levels were found in fat, with more than 10 times higher levels compared to other tissue residue levels. A comparative distribution and clearance study with avermectin B_{1b} following single oral doses showed that the toxicokinetic profile was essentially the same as that of avermectin B_{1a}.

5.1.3 Metabolism

The metabolite pattern in urine, faeces and bile is complex, and 11 metabolites were isolated. In faeces, avermectin B_{1a} accounted for 24 to 45% of the dose, and the metabolite 3''-O-desmethyl-avermectin B_{1a} [=3''DM] accounted for 19-27%. These major faecal components were not present in urine. In fat and muscle, avermectin B_{1a} was the major component (92% and 72%, respectively), and metabolite [3''DM] accounted for 1.7% and 19% in the fat and muscle, respectively. The major reactions involved in the biotransformation of avermectin B_{1a} in the rat are demethylation, hydroxylation, cleavage of the oleandrosyl ring and oxidation reactions.

In rat the 8,9-Z isomer of abamectin B1a is not formed.

5.1.4 Excretion

Avermectin B_{1a} and/or metabolites is rapidly eliminated from the body, almost exclusively in the faeces (more than 92% of the dose within 7 days, urinary excretion accounting for 0.9-1.6% of the dose in males and 0.5-1.0% in females of low and high dose groups). Initially, the rate of excretion was slower in females as compared to males. The excretion via expired air accounted for only 0.01% of the dose within 48h after administration. Tissue half-lives were mostly within the range of 1.2 ± 0.3 days, with the tissue half-lives of avermectin B_{1a} being lower in males (12 to 17 h) compared to females (13 to 33 h). So, with the exception of dose-dependence for tissue residue levels and excretion by urine, the toxicokinetic profile is not influenced by sex, dose level or treatment regime.

5.1.5 Summary and conclusion

Table 5.1-1 summarises toxicokinetics of abamectin in rats and humans.

Absorption	Oral: Complete oral absorption with a calculated bioavailability of 0.86. For risk assessment of abamectin a value of 100% is assumed. Inhalation: No data are available. Absorption is assumed to be 100%. Dermal: less than 1% absorption.
Distribution	Widely distributed
Metabolism	Extensive metabolism (demethylation, hydroxylation, cleavage of oleandrosyl ring and oxidation reactions).
Excretion	Rapidly eliminated from body, almost exclusively in faeces.

CONCLUSION

Abamectin is almost completely absorbed in the gastrointestinal tract of the rat (calculated oral bioavailability is 0.86) and distributed throughout tissues and organs. It is rapidly eliminated from the body, almost exclusively in the faeces, and does not accumulate in tissues/organs after repeated exposure. The major reactions involved in the biotransformation of abamectin in the rat are demethylation, hydroxylation, cleavage of the oleandrosyl ring and oxidation reactions.

Dermal penetration is very low, less than 1% is absorbed through the skin of monkeys. For the inhalation route 100% absorption is assumed.

5.2 Acute toxicity

5.2.1 Acute oral toxicity

Animal data

Abamectin is very toxic to the rat by oral administration in sesame oil (LD_{50} 8.7-12.8 mg/kg bw). However, a subsequent study with an aqueous vehicle showed that abamectin was significantly less toxic orally with this vehicle. In the toxicokinetic studies performed with sesame oil or polyethylene glycol there are no indications for this observed difference in toxicity. Characteristic signs of abamectin toxicity after oral administration are tremors and ataxia. As abamectin is lipophilic the sesame oil is considered to be a more suitable vehicle than water, and classification of abamectin for acute toxicity will be based on the LD_{50} values from the studies in which sesame oil was used as vehicle.

In an acute neurotoxicity study in rats abamectin, administered by gavage induced clinical signs of neurotoxicity, i.e. reduced splay reflex, tiptoe gait and splayed gait. The NOAEL was 0.5 mg/kg bw, based on reduced splay reflex at 1.5 mg/kg bw. At 6 mg/kg bw, reduced splay reflex, tip toe and splayed gait and a transient reduction in motor activity was observed.

Human data

Available human data from suicide attempts show that typical clinical signs of abamectin toxicity in animal studies, like tremors and convulsions, do not occur in humans. No signs of poisoning were reported in a few cases after ingestion of low doses (up to 40 mg/kg bw). In other cases (estimated exposure 4.2 - 67 mg/kg bw), nausea, vomiting and diarrhoea or short lasting CNS depressions like dizziness, drowsiness and weakness were observed. Severe poisoning after suicidal ingestion of high amounts of an abamectin formulation (equivalent to 38.5 - 227.3 mg/kg bw abamectin) resulted in a comatose state within 3 hours after ingestion, shock, respiratory failure and even death as a result of multiple organ failure. The dose of abamectin ingested orally by a patient with lethal outcome in suicidal intention was 88.1 mg/kg. The maximum tolerated dose via the same route by another patient was 227.3 mg/kg.

5.2.2 Acute inhalation toxicity

Two acute inhalation toxicity studies with rats are available and included in this report.

Characteristics

Reference/notifier	:	Ruddock, W. (2001a)	Exposure	:	4 h (nose only)
Type of study	:	Acute inhalation toxicity study	Dose	:	0.21, 1.78 and 5.25 mg/L (MMAD 4.2, 3.7 and 2.7 resp., GSD 3.8, 4.9 and 3.4 resp.)
Year of execution	:	2001	Vehicle	:	-
Test substance	:	Abamectin (purity 89.3% and 96.7%)	GLP statement	:	yes
Route	:	inhalation	Guideline	:	In accordance with OECD 403
Species	:	Rat (Crl:Han Wist)	Acceptability	:	acceptable
Group size	:	5/sex/dose	LC_{50} rats	:	< 0.21 mg/L

Study design

The study is in accordance with OECD 403.

Results

Mortality: There was 100% mortality in all dose groups. Death or moribund sacrifice occured during exposure or within 2 h for the 5.25 mg/L animals, within 5 h for the 1.78 mg/L animals and by the day following exposure for the 0.21 mg/L animals.

Symptoms of toxicity: tremors, rigid tail and prostrate body position were observed in all animals, whereas signs observed in some animals included ataxia, cyanosis, subdued behaviour, piloerection, noisy respiration, coloured tears, staining of the eye and fur, squinting, wet fur, vocalisation on handling and tail flicking.

Body weight: Body weight analysis was not appropriate due to the early termination of the rats.

Pathology: dark, darkened or red areas in the lungs were observed in all dose groups, inflated lungs and firmness along the length of the tail was noted for all 5.25 mg/L animals.

Acceptability

The study is considered acceptable.

Conclusions

The acute 4-hour inhalatory LC_{50} in rats is <0.21 mg/L.

Characteristics

Reference/notifier	:	Noakes, J.P. (2003)	Exposure	:	4 h (nose only)
Type of study	:	Acute inhalation toxicity study	Dose	:	m + f: 0.051 mg/L (MMAD 2.11, 2.29; GSD 1.69, 1.83); f: 0.034 mg/L (MMAD 2.80, 2.57; GSD 1.73, 1.70)
Year of execution	:	2003	Vehicle	:	-
Test substance	:	Abamectin (purity 88.3%)	GLP statement	:	yes
Route	:	inhalatoir	Guideline	:	In accordance with OECD 403
Species	:	Rat (Alp:APfSD)	Acceptability	:	acceptable
Group size	:	5/sex (0.05 mg/L) and 5f (0.03 mg/L)	LC_{50} rats	:	>0.051 mg/L (m); >0.034 mg/L and <0.051 mg/L (f)

Study design

The study is in accordance with OECD 403, with the following deviation: there are only 2 concentrations tested, and exposure to 0.03 mg/L was performed with 5 females only, pathology was not performed.

Results

Mortality: In the 0.05 mg/L group, one female was found dead and 2 females were killed on day 2 due to the severity of the clinical signs on day 2.

Symptoms of toxicity: reduced splay reflex, prostrate and tip toe gait, shaking, comatose, increased response to touch, reduced stability, decreased visual placing response, abnormal respiratory noise, increased breathing depth were observed in animals of the 0.051 mg/L group. In the 0.034 mg/L group, abnormal respiratory noise was observed staining of the oral and nasal cavities and eye discharge, wet fur, hunched posture, piloerection and chromodacryorrhea. Full recovery was apparent by day 4 for surving females and for males by day 15.

Body weight: normal

Pathology: not performed

Acceptability

The study is considered acceptable.

Conclusions

The acute 4-hour inhalatory LC_{50} in rats is >0.051 mg/L for males and between 0.051mg/L and 0.034 mg/L for females.

5.2.3 Acute dermal toxicity

Topical application of abamectin resulted in the rabbit in a 24hr LD_{50} value >2000 mg/kg and in the rat in a 24hr LD_{50} value >330 mg/kg (highest dose tested). The low order of toxicity by topical application indicates a low order of percutaneous penetration. This is supported by data in rhesus monkeys which demonstrate that < 1% of the applied dose is absorbed through the skin in to the systemic circulation.

Characteristic signs of abamectin toxicity, tremors and ataxia, occur in rats at 330 mg/kg bw about 3 days after administration and in rabbits at 2120 mg/kg bw within 6 days after administration.

5.2.4 Acute toxicity: other routes

No data

5.2.5 Summary and discussion of acute toxicity

Table 5.2-1 Key acute toxicity (LD_{50}/LC_{50}) studies reported for abamectin

Route	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}/LC_{50}	Reference
Oral	Not fully in compliance with OECD 401 (but rated acceptable in 91/414/EEC DAR)	Rat/ CRCD 10 m/f	6.67, 10, 15, 22.5, 33.75 mg/kg bw in sesame oil single dose	M: 8.7 F: 12.8	Robertson 1981f
Oral	In compliance with OECD 401	Rat/- 5 m/f	20, 50, 100, 275, 500 mg/kg bw in 0.5% methylcellulose in water single dose	M: 232 F: 214	Glaza 2001
Dermal	Not fully in compliance with OECD 402 (but rated acceptable in 91/414/EEC DAR)	Rat/CD(SD)BR 5 m/f	330 mg/kg bw; 24h exposure	> 330	Gordon 1985a
Dermal	Not fully in compliance with OECD 402 (but rated acceptable in 91/414/EEC DAR)	Rabbit 5 m/f	2120 mg/kg bw; 24h exposure	> 2000	Gordon 1984a
Inhalation	In compliance with OECD 403	Rat/Crl:Han Wist	0.21, 1.78 and 5.25 mg/L 4 h (nose only)	<0.21	Ruddock, W. (2001)
Inhalation	Not fully in compliance with OECD 403 (but rated acceptable in 91/414/EEC DAR)	Rat/Alp:Ap$_f$SD) 5 m/f	0.051 mg/L (m/f) 0.034 mg/L (f) 4 h (nose only)	M: >0.051 F: between 0.034 and 0.051	Noakes 2003

Table 5.2-2 Summary of acute neurotoxicity study

Species	Study type or duration; Dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effect at LOAEL	Reference
Rat	Oral study of acute neurotoxicity, OECD 424. 0, 0.5, 1.5, 6 mg/kg bw by gavage	0.5	1.5	reduced splay reflex	Brammer, A. 2006a

Classification proposals according to Directive 67/548/EEC

Based on the acute oral LD_{50} values (8.7-12.8 mg/kg bw) observed in the rat, which are below the threshold value of 25 mg/kg/day for oral acute toxicity (T+; R28), abamectin needs to be classified as T+; R28 "very toxic if swallowed". Based on the acute inhalation LC_{50} value (<0.21 mg/l; females 0.034-0.051 mg/l, males 0.051-0.21 mg/l), which is lower than the threshold value of 0.25 mg/l/4h for acute inhalation toxicity (T+; R26) of particulates, abamectin needs to be classified as T+; R26 "very toxic by inhalation". Based on the available data the compound needs not to be classified when exposed via skin.

The limited number of human cases seems to indicate a somewhat lower acute oral toxicity of abamectin towards humans (lethality at 88 mg/kg bw/day) compared to rats. However, most patients were intensively treated. Also, it is unclear whether the vehicle has affected the human toxicity. The rat data are considered relevant for humans in a quantitative way.

Classification proposals according to Regulation EC 1272/2008

According to CLP criteria, and based on the data mentioned above, abamectin should be classified in acute hazard category 2 for oral exposure (threshold values 5-50 mg/kg/day) and in acute hazard category 1 for inhalation exposure (threshold values $\leq$ 0.05 mg/l for particulates), and labelled with signal word 'Danger' and hazard statements: H300 and H330 respectively.

Clinical signs of mild neurotoxicity were observed in an acute oral (gavage) neurotoxicity study at 1.5 and 6 mg/kg bw. It is noted that this is not much lower than the LD_{50}s of 8.7-12.8 mg/kg bw (on which basis it is proposed to classify abamectin in acute hazard category 2 for oral exposure).In humans ingestion of doses up to 40 mg/kg bw induced no signs of poisoning.

Mild signs of neurotoxicity were observed in animals while in human poisoning cases with relative low doses no neurotoxic effects were reported. Since it is already proposed to classify abamectin for acute toxicity on the basis of the LD_{50} studies, no additional classification of abamectin for Specific Target Organ Toxicity-Single Exposure (STOT-SE) is necessary.

5.3 Irritation

5.3.1 Skin Irritation

In a study with rabbits, abamectin did not cause any irritation of the skin (table 5.3-1).

Table 5.3-1 Skin Irritation

Species	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference
		Erythema	Oedema			
Rabbits; New Zealand White	Not fully in compliance with OECD 404 (but rated acceptable in 91/414/EEC	0, 0, 0	0, 0, 0	-	negative	Robertsen (1981b)

5.3.2 Eye irritation

In a study with rabbits, abamectin did not cause any irritation of the eyes (table 5.3-2).

Table 5.3-2 Eye irritation

Species	Method	Average Score 24, 48, 72 h				Result	Reversibility yes/no	Reference
		Cornea	Iris	Conjunctiva Redness	Chemosis			
Rabbits; New Zealand White	in accordance with OECD 405	0	0	0	0	Not irritating	-	Glaza (2000)

5.3.3 Respiratory tract

In an acute inhalation toxicity study (Ruddock, 2001) dark, darkened or red areas in the lungs and inflated lungs were observed in all dose groups (0.21-5.25 mg/L).

5.3.4 Summary and discussion of irritation

Classification proposals according to Directive 67/548/EEC

Abamectin is considered not irritating to skin or eyes according to the criteria of Annex VI of Directive 67/548/EC.

The description of the effects on the lung in the acute toxicity study is limited. Since in repeated dose studies histological examination revealed no signs of respiratory irritation and since abamectin are not irritating to the eyes and the skin no classification for respiratory irritation is required.

Classification proposals according to Regulation EC 1272/2008

Abamectin also needs not to be classified for skin and eye irritation according to the criteria in the new EU C&L Regulation based on GHS. For reasons described above abamectin needs not to be classified for respiratory irritation.

5.4 Corrosivity

Based on the data from the skin irritation study it can be concluded that abamectin is not corrosive.

5.5 Sensitisation

5.5.1 Skin

Abamectin showed no skin sensitizing properties in a Guinea Pig Maximization test (table 5.5-1).

Table 5.5-1 Sensitisation

Species	Method	Number of animals sensitised/total number of animals	Result	Reference
Guinea Pig	GPMT in accordance with OECD 406	0/19	negative	Ruddock (2001b)

5.5.2 Respiratory system

No data.

5.5.3 Summary and discussion of sensitisation

Abamectin needs not to be classified for skin or respiratory sensitization according to Directive 67/548/EC.

Abamectin needs not to be classified for skin or respiratory sensitization according to Regulation EC 1272/2008.

5.6 Repeated dose toxicity

No repeated exposure toxicity data in humans are available.

5.6.1 Repeated dose toxicity: oral

In the rat an 8-week and a 90-day dietary study were performed. In the dog 12, 18 and 53-week toxicity studies have been performed by dietary, gavage and dietary administration respectively. The studies were performed using abamectin except the 18 week toxicity study in dogs which used avermectin B_{1a}. The 8-week study in the rat and the 12 week study in the dog were range finding studies, with determination of very few parameters, not in accordance with OECD guidelines.

In a 90-day study of (neuro-)toxicity in rats abamectin, administered daily by gavage at 4 mg/kg bw/day induced low incidences of clinical signs from week 2 onwards. In these animals a marked increase in clinical signs (shaking, tiptoe gait, reduced righting reflex, reduced stability, reduced splay reflex, hunched posture, "pinched-in" sides, subdued behaviour, irregular breathing, decreased activity, stains around the mouth or nose, upward spinal curvature) and body weight loss occurred in week 7 of treatment. The animals were killed for humane reasons. Pathological examinations revealed macroscopic and histological changes in the stomach. The NOAEL in this study was 1.6 mg/kg bw/day. The LOAEL was 4.0 mg/kg bw/day.

In the 18 week oral toxicity study with dogs, a very steep dose-response relationship for avermectin B_{1a} in the dog was observed, since the oral NOAEL by gavage is 0.25 mg/kg bw/day and death, clinical signs (ataxia, tremors, mydriasis and ptyalism), reduced weight gain and histopathological changes in the liver occurred at 0.5 mg/kg bw/day. At the highest dose these effects were observed after the first dose. At the lower dose levels, the effects were observed after several exposures.

In the 53-week oral toxicity study with abamectin in dogs, death occurred at the high dose level of 1.0 mg/kg bw/day, and pupil reactivity was decreased or absent at the dose level of 0.5 mg/kg bw/day. Based on this effect on pupil reactivity, the NOAEL in this study is 0.25 mg/kg bw/day. The results of both these studies show that a similar steep dose response exists for abamectin .

The overall NOAEL in the short-term toxicity studies is 0.25 mg/kg bw/day for both abamectin and avermectin B_{1a}, observed in an 18-week and a 1-year study in the dog.

Table 5.6-1 Summary of neurotoxicity studies

Species	Study type or duration; Dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effect at LOAEL	Reference
Rat	90-day oral study of (neuro-)toxicity; OECD 408, OECD 424. 0, 0.4, 1.6, 4 mg/kg bw/day	1.6	4	clinical signs (shaking, tiptoe gait, reduced righting reflex, reduced stability, reduced splay reflex, hunched posture,	Brammer, A. 2006b

	by gavage				"pinched-in" sides, subdued behaviour, irregular breathing, decreased activity, stains around the mouth or nose, upward spinal curvature) and body weight loss and macroscopic and histological changes in the stomach	

Chronic toxicity

Long-term toxicity and carcinogenicity studies were performed in the rat and the mouse. There was no evidence of carcinogenicity in either the rat or the mouse at any of the dose levels employed. The long-term dietary administration of abamectin did not reveal any primary target organ toxicity. Although clinical signs of neurotoxicity were evident in rats and to a lesser extend in mice, no histopathologic correlate was evident. The overall NOAEL determined in long-term toxicity studies was 1.5 mg/kg bw/day found in the rat carcinogenicity and toxicity study.

Table 5.6-2 Summary of repeated dose toxicity studies

Test substance	Duration, route Dose levels	Species	NOAEL (mg/kg bw/day	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Registrant
Abamectin (vehicle acetone)	4 or 8 weeks, oral 0, 5, 10, 15, 20/25, 40 (and 60) ppm (mean weekly achieved dose 0, 0.3-0.7, 1.0-1.4, 1.6-2.2, 1.7-2.7 and 4.1-5.8 mg/kg bw/day)	rat	-	-	Range-finding study (only bw, food consumption and clin. signs)	Gordon, L.R. (1984b)
Abamectin (acetone)	12 weeks, oral 0, 0.25, 0.5, 1.0 and 4.0/2.0 mg/kg (0, 6, 13, 25, 100/50 ppm)	dog	(0.5)	(1.0)	Range-finding study (only bw, food consumption and pupil response)	Gordon, L.R. (1984c)
Avermectin B_{1a} (vehicle sesame oil)	18 weeks, oral (gavage) 0, 0.25, 0.5, 2.0 and 8.0 mg/kg bw/day	dog	0.25	0.5	Mortality, clinical signs of toxicity (ataxia, tremors, mydriasis, ptyalism), reduced weight gain, histopathologic changes in the liver	Robertson , R.T. & Allen, H.L. (1976)
Abamectin (vehicle acetone)	53 weeks, oral (diet) 0, 0.25, 0.5 and 1.0 mg/kg bw/day	dog	0.25	0.5	Absent or decreased pupil reflex (death at 1.0 mg/kg bw/day)	Gordon, L.R. (1984d)
Abamectin (vehicle acetone)	105 weeks, oral (diet) 0, 0, 0.75, 1.5 and 2.0 mg/kg bw/day	Rat	1.5	2.0	Increased mortality in males, clinical signs (tremors, unthrifty appearance)	Gordon, L.R. (1985b)
Abamectin (vehicle acetone)	94 weeks, oral (diet) 0, 0, 2.0, 4.0 and 8.0 mg/kg bw/day	CD-1 mice	4.0	8.0	Increased mortality in males, reduced body weight gain in males and females, extramedullary haematopoiesis in spleen of males.	Gordon, L.R. (1985c)

5.6.2 Repeated dose toxicity: inhalation

In a preliminary study in rats (2/sex/dose) daily inhalation exposure for 5 consecutive days induced dose-dependent increases in clinical signs after exposure at all doses (1.03-24.7 µg/L). The severity of the clinical signs was such that at 9.59 and 24.7 µg/L (part of) the animals were humanely killed during the treatment period. Pathological examination revealed no relevant macroscopic or microscopic effects.

A repeated dose inhalation study was described in the addendum to the DAR (February 2008). Daily inhalation exposure (nose only) of rats for 6 h/day, 5 days/week over a 30 day period (total of 21 exposures) induced clinical signs and reduced motor activity at 2.69 µg/L. The NOAEC was 0.577 µg/L.

Pathological examination revealed no relevant macroscopic or microscopic effects.

Table 5.6-3 summarises the repeated dose inhalation toxicity study.

Table 5.6-3 Summary of repeated dose inhalation toxicity study

Test substance	Duration, route Dose levels	Species	NOAEC (µg/L)	LOAEC (µg/L)	Critical effects	Reference/ Registrant
Abamectin	6h/day, for 5 days. Inhalation (nose only) 0, 0.103, 3.71, 9.59 and 24.7 µg/L.	Rat			Dose-dependent increase in number and severity of clinical signs (splay reflex, hunched posture reduced foot splay reflex, tremors, decreased activity, piloerection, shaking, reduced stability,pale skin, tail erection, reduced breathing rate, decreased visual placing response), and body weight loss and reduced food consumption	Pinto, P.J. (2006a)
Abamectin	6h/day, 5 days/week over a 30-day period. Inhalation (nose only) 0, 0.111, 0.577 and 2.69 µg/L.	rat	0.577	2.69	Clinical signs (lying prostrate, shaking and gasping, with a swollen head. Ungroomed appearance, with stains around the mouth, hunched posture and piloerection, abnormal respiratory noise) and decreased motor activity.	Pinto, P.J. (2006b)

5.6.3 Repeated dose toxicity: dermal

No repeated dose studies were available.

Acute dermal toxicity studies with rat and rabbit has shown that abamectin has a low order of toxicity. A dermal penetration study with monkeys has shown that less than 1% of abamectin is absorbed through the skin. Based on these findings, percutaneous exposure will not be a significant route of exposure.

5.6.4 Other relevant information

None.

5.6.5 Summary and discussion of repeated dose toxicity:

Effect:

With respect to oral exposure, based on the severity of clinical signs of neurotoxicity and mydriasis and the dose levels at which death occurs, the dog is more sensitive than the rat to abamectin. Repeated dose dietary administration of abamectin reveals that the nervous system is a primary target organ for toxicity. A steep dose response curve exists for this effect. Although clinical signs of neurotoxicity occur in all species evaluated, no histopathologic correlates are evident in central or peripheral nerves. In addition, histopathologic changes in the liver of dogs and extramedullary haematopoiesis in the spleen of mice were observed.

With respect to inhalation toxicity, the data in the rat study indicate that the nervous system is the primary target organ for toxicity. The mechanism for repeated dose toxicity and acute toxicity are both likely involving GABA-antagonism-induced neurotoxicity. However, the inhalation exposure levels needed to exert these effects differ, with 0.00269 mg/l being LOAEC after repeated exposure and 0.051 mg/l for acute lethal effects, thus a 19-fold difference in effective concentration. It is therefore relevant with classification also for repeated dose toxicity.

Dose:

The overall NOAEL in oral repeat dose toxicity studies is 0.25 mg/kg bw/day for both abamectin and avermectin B1a, observed in an 18-week and a 1-year study in the dog. This about 30 times lower than the acute oral LD_{50}.

The overall NOAEC in inhalation repeat dose toxicity studies is 0.577 µg/L for abamectin observed in a 30-day study in rats. The LOAEC was 2.69 µg/L. This is about 20 times lower than the acute LC_{50}.

Relevant repeated exposure effect levels for classification

Clear signs of oral neurotoxicity were observed in a 90-day study in rats at a dose of 4 mg/kg bw/day. In an 18-week oral (gavage) study in dogs severe signs of toxicity, including mortality, were observed at 0.5 mg/kg bw/day. In a 2-year dietary study in rats severe signs of toxicity, including mortality, were observed at 2.0 mg/kg bw/day. Clear signs of neurotoxicity were also observed in a 30-day inhalation study (6h/day, 5 days/week) in rats, with a LOAEC of 2.69 µg/L (=0.00269 mg/L).

Classification proposal according to Directive 67/548/EEC

In view of the effects and effect levels for oral and inhalation (neuro-)toxicity in repeated exposure studies abamectin should be classified with R48/23/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation and if swallowed. There was clear neurotoxicity at 0.00269 mg/L which is below the guidance value for R48/23 in a 30 day inhalation study of 0.075 mg/L. In the oral 18-weeks dog study, neurotoxicity and mortality were observed at 0.5 mg/kg/day, a dose level clearly below the guidance value for R48/25 of 5 mg/kg bw/day in a 13-week study.

Classification proposal according to Regulation EC 1272/2008

In oral repeated dosing studies in animals abamectin appears to be (neuro-)toxic at doses of 4 and 0.5 mg/kg/day in rats and dogs, respectively, which is lower than the threshold value of < 10 mg/kg bw/day for oral 13-week studies. In a 30-day repeated exposure inhalation study in rats, abamectin is neurotoxic at concentrations of 0.00269 mg/L and above (range-finding study). This is below the guidance value for STOT-RE Cat 1 in a 30 day inhalation study of 0.06 mg/L (particulates). According to CLP criteria, abamectin should be classified with **STOT-RE Cat. 1, H372**, with the hazard statement "**Causes damage to the nervous system through prolonged or repeated exposure**". No specific route of exposure is stated, as the acute toxicity studies show that all routes of exposure may cause adverse effects.

In relation to the criteria both in Directive 67/548/EEC and Regulation (EC) 1272/2008, it is acknowledged that a 18-week oral study in dogs is compared with thresholds for 13-week studies. However, considering the severity of effects in the 18-week study and how much lower the 18-week effect level is than the 13-week threshold level, the substance is judged to clearly fulfil the criteria for R48/25 and STOT-RE Cat. 1, H372.

Specific concentration limits (SCL)

According to the CLP guidance, specific concentration limits for repeated dose toxicity are needed if the effective dose is more than a magnitude lower than the guidance value. This applies for inhalation exposure, where the 30-day LOAEC (0.00269 mg/l) should be compared with the 30 day guidance value of 0.06 mg/l, giving a SCL of 4.5%, which is rounded off to 5%, for triggering classification of preparations/mixtures as T;R48/23 (STOT-RE Cat. 1; H372). Correspondingly, the SCL for triggering classification of preparations/mixtures as Xn;R48/20 (STOT-RE Cat. 2; H373) is 0.5%. The corresponding generic concentration limits are 10% and 1%.

The SCL:s set for repeated dose toxicity by the inhalation route will be applied under both Directive 67/548/EEC and Regulation (EC) 1272/2008.

5.7 Mutagenicity

5.7.1 In vitro data

Table 5.7-1 summarises in vitro genotoxicity studies. Abamectin does not induce gene mutations in either bacteria or mammalian cells with or without metabolic activation. There is no evidence of clastogenicity in an in vitro test system.

Table 5.7-1 Genotoxicity studies: In vitro

Test system Method Guideline	organism/ strain(s)	concentrations tested (give range)	Result + S9	 - S9	Remark give information on cytotoxicity and other	Reference
Point mutation OED 471	S. typhimurium (5 strains)	3, 10, 30, 100, 300, and 1000 µg/plate	neg	neg		Gordon L.R. (1986a)
Point mutation OED 471	S. typhimurium (5 strains) & E.coli (1 strain)	312.5, 625, 1250, 2500, 5000 µg/plate	neg	neg		Deparade, E (2001)
Chromosome aberrations (in vitro) OECD 473	Chinese hamster ovary cells (CHO-WBL)	-S9: 0.0100, 0.0150, 0.0200, 0.0250, 0.0300 and 0.0350 mM +S9: 0.0050, 0.0100, 0.0150, 0.0200 and 0.0250 mM Solvent: DMSO	neg	neg		Gordon L.R. (1986b)

Mammalian point Mutations OECD 476	Chinese hamster lung cells (V79)	-S9[1]: 0.003, 0.004, 0.005 and 0.006 mM +S9: 0.03, 0.04, 0.045 and 0.05 mM Solvent: DMSO	neg	neg	Gordon L.R. (1983)

[1] Due to a dilution error, the two lowest concentrations tested without S9 in the repeat assay were 0.0003 and 0.0004 mM

5.7.2 In vivo data

Table 5.7-2 summarises in-vivo genotoxicity studies. Abamectin does not induce cytogenic damage in male mouse bone marrow cells.

Table 5.7-2 Genotoxicity studies: In vivo

Test system Method Guideline	species strain(s)	Dose levels tested (give range)	Result	Remark give information on cytotoxicity and other	Reference
Structural chromosome aberration OECD 475	Mouse (male CD-1)	0, 1.2, 4.0 and 12 .0 mg/kg bw	negative		Blazak, WF (1983)

5.7.3 Human data

No data available.

5.7.4 Other relevant information

None.

5.7.5 Summary and discussion of mutagenicity

5.7.6 Conclusion

Abamectin did not induce gene mutations in either bacterial or mammalian cells at any of the tested concentrations either with or without metabolic activation. There was no evidence of a clastogenic effect at any tested concentration either in vitro or in vivo. It is concluded that abamectin and/or its metabolites are not genotoxic.

Abamectin needs not to be classified for mutagenicity according to Directive 67/548/EEC or Regulation EC 1272/2008.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

Table 5.8-1 summarises carcinogenicity studies. Long-term toxicity and carcinogenicity studies were performed in the rat and mouse. There was no evidence of carcinogenicity in either the rat or the mouse at any of the dose levels employed.

Table 5.8-1 Carcinogenicity study

Route	Species Strain Sex no/group	dose levels frequency of application	Tumours	Reference
Oral in diet	Rat (CD(SD)BR)	105 weeks, oral 0, 0, 0.75, 1.5 and 2.0 mg/kg bw/day	none	Gordon, LR (1985b)
Oral in diet	Mouse (CD-1)	93 weeks, oral 0, 0, 2.0, 4.0 and 8.0 mg/kg bw/day	none	Gordon, LR (1985c)

5.8.2 Carcinogenicity: inhalation

No data available.

5.8.3 Carcinogenicity: dermal

No data available.

5.8.4 Carcinogenicity: human data

No data available.

5.8.5 Other relevant information

None.

5.8.6 Summary and discussion of carcinogenicity

Abamectin is unlikely to pose a carcinogenic hazard.

Abamectin needs not to be classified for carcinogenicity according to Directive 67/548/EEC or to Regulation EC 1272/2008.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

A rat 2-generation reproductive toxicity study with abamectin was available. For the plant protection evaluation the registrant provided additional data to the study report (see revised addendum to pesticides Draft Assessment Report, February 2008).

The original study report did not include all relevant parameters. Furthermore, the study report incorrectly suggested that in this study fertility of the rats was affected by abamectin treatment. However, the registrant provided additional information on the fertility study and recalculated some reproduction parameters. The additional information and the recalculated reproduction parameters were evaluated by the rapporteur in 2008. The conclusion of the re-evaluation of this 2-generation reproductive toxicity study was reported in an addendum to the DAR. The results of final evaluation of the study are summarized in table 5.9-1.

Table 5.9-1 Results of two-generation study in rats

Dose (mg/kg bw/day)		0		0.05		0.12		0.4		dr
		m	f	m	f	m	f	m	f	
F0 animals										
	Mortality	No treatment-related deaths								
	Clinical signs	No toxicologically relevant effects								
	Body weight gain -during lactation F1a -during lactation F1b					i	is d d	is	is ds d	mf f
	Food consumption	Not performed								
	Water consumption	Not performed								
	Sperm parameters	Not performed								
	Testes and epididymides weight	No toxicologically relevant effects								
	Pathology	Not performed								
	macroscopy									
	microscopy									
Effect during F1a mating		No toxicologically relevant effects								
Effects during F1b mating		No toxicologically relevant effects								
F1 pups										
Effects on F1a litters	-pup mortality (%), days 5-15	6.3		2.7		3.0		42.2 (is)		
	-pup weight (days 7-21)							ds		
	-incidence of total litter loss (%)	8.0		3.8		0.0		28.0 (is)		
	-lactation index [a](% survival day 4-21)	99.5		100		99.2		52.7 (ds)		
Effects on F1b litters	-pup mortality (%), days 5-15	2		2		4		33 (is)		
	-pup weight (days 7-21)							ds		
	-incidence of total litter loss (%)	0.0		0.0		0.0		25.0 (is)		
	-lactation index [a](% survival day 4-21)	98.0		98.5		99.2		60.0 (ds)		
	Clinical signs F1a and F1b -thin and not nursing							i		
	Sex ratio	No toxicologically relevant effects								
	Skeletal evaluation	No toxicologically relevant effects								
	Retinal anomaly							i		
F1 animals										
	Mortality	No treatment-related deaths								
	Clinical signs	No toxicologically relevant effects								
	Body weight					ds		ds	ds	
	Food consumption	Not performed								
	Water consumption	Not performed								
	Oestrus cycle	Not performed								
	Sperm parameters	Not performed								
	Organ weights	No toxicologically relevant effects								
	Pathology									
	macroscopy									
F2a pups	Pup mortality (%)	1.8		1.3		1.2		6.7 (is)		
	Body weight/litter, day 7-21							ds		
	Male pups/litter, day 1 (%)	58		55		52		46		dr
	Lactation index							ds		

		0	0.05	0.12	0.4	dr
	Viability index (day 4-14)				ds	
	Gross litter observation -thin -weak -not nursing				 i i i	
F2b pups	Pup mortality (%)	4.2	1.6	1.5	8.6	
	Body weight/litter, day 7-21				ds	
	Male pups/litter, day 1 (%)	58	50	53	46	
	Lactation index				ds	
	Viability index (day 4-14)				ds	
	Gross litter observation -thin -weak -not nursing				 i i i	
	pathology microscopy - retinal anomaly				 is is	

It was concluded that in this multigeneration reproductive toxicity study in the rat the NOAEL for parental and reproduction toxicity is 0.4 mg/kg bw/day, i.e. the highest dose tested (see revised addendum to pesticides Draft Assessment Report, February 2008).

In the 2-generation study, pup mortality for both F1a and F1b litters was significantly increased at 0.4 mg/kg bw/day, with most pups dying days 5-15 postpartum. Post mortem examination of F1b weanlings showed retinal anomalies (single or multiple retinal folds of many layers of the retina) in 3 out of 4 males in the highest dose group.

Group mean body weights of F1 males and females at 0.4 mg/kg bw/day and the females at 0.12 mg/kg bw/day were significantly reduced at the start of treatment, due to retarded pre-weaning growth. Treatment-related reduced weight gain continued in males at 0.4 mg/kg bw/day for 4 weeks, after which weight gain was enhanced and terminal body weights were comparable to controls. This temporary effect on body weight is considered not a relevant endpoint for determination of the LOAEL. Retinal anomaly was observed in pups only, and appeared to be transient, and was not observed in the adult F1 animals.

In both F2a and F2b litters treated at 0.4 mg/kg bw/day pup mortality significantly increased during the course of lactation, and the associated viability and lactation indices significantly decreased. Pup weight in the high dose group was unaffected by treatment directly after birth and for the first few days, but was significantly reduced from day 7 to day 21. This was associated with increased numbers of pups that were thin, weak and not nursing. The number of male pups was decreased in the high dose group (F2a andF2b). Post mortem examination of F2b weanlings showed retinal anomalies, with characteristics identical to those observed in F1b animals in 10/63 males and 18/66 females in the highest dose group. As in the F1 pups, it is considered that these retinal anomalies are are transient and confined to the pup stage.

Based on the occurrence of increased pup mortality and retarded weight gain in both F1 and F2 generation progeny, increased incidence of total litter loss, decreased lactation index and reduced weight gain in the F1 and F2 generation weanlings at the highest dose, the NOAEL for pup toxicity in this study is 0.12 mg/kg bw/day.

Significant neonatal mortality seen in rat pups is likely to be the result of a lack of p-glycoprotein expression in the neonatal rat brain. P-glycoprotein dependent xenobiotic efflux in the blood brain barrier is considered to play an important role in attenuating neurotoxicity of avermectins. However, brain p-glycoprotein expression starts early in human development, having been detected in human foetal brain microvessels as early as week eight of pregnancy. Expression of p-glycoprotein in the cerebrum and cerebellum of rats is not fully developed in neonate rats and expression in the jejunal epithelial brush borders does not start before post-natal day 8. Adult levels are reached at post-partum day 20 or 28. Therefore, the increased postnatal mortality is considered not to be relevant to human risk assessment and also not for classification and labelling. For further information on the role of p-glycoproteins in abamectin toxicity see section 5.10.1.

Table 5.9-2 Fertility study

Species	Study type or duration; Dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effect at LOAEL	Reference
rat	Two-generation study in rats with abamectin ; OECD 416. 0, 0.05, 0.12 and 0.4 mg/kg bw/day	0.4	--	**Parent**: No effects	Gordon, LR (1984e)
		0.4	--	**Reproduction**: No effects	
		0.12	0.4	**Fetes/pups**: increased postnatal pup mortality, retarded weight gain pups (F1 and F2), increased incidence of total litter loss, decreased lactation index, increased incidence of retinal anomaly in the eyes of pups (F1 and F2)	

5.9.2 Developmental toxicity

Two developmental toxicity studies (rat and rabbit) with abamectin were available.

Rats

In a teratogenicity study in rats the sex ratio (m:f) was lower at 1.6 mg/kg bw/day. Since exposure to abamectin was from days 6-19 of gestation, abamectin could not have affected the sex of the fetuses directly. Apparently, abamectin exposure in the highest dose group affected resorption in a sex-specific way (more effect on female fetuses), resulting in a lower m:f ratio but within the historical control range of 1 : 0.69 to 1 : 1.22. However, there was no increase in the total number of resorptions.

In the 0.8 mg/kg bw/day group a significant higher incidence of resorptions and decreased fetal weight were observed. Similar effects were not observed at 1.6 mg/kg bw/day, and therefore these effects are considered incidental. At 1.6 mg/kg bw/day, exencephaly was observed paired with a conjoined twin, which is a spontaneous congenital abnormality, and thus it is likely that this effect is not substance-related. The observed incidence of one animal with cleft palate in the highest dose groups is considered treatment-related, since this effect is also observed in the developmental toxicity study with abamectin in the rabbit and in the developmental toxicity study with the main isomer of abamectin in CD-1 strain mice. Furthermore, historical control data provided by the registrant in 2005 showed that in 23 studies only one fetus with cleft palate was observed.

In the highest dose group, the number of pups with lumbar rib and with lumbar count variation had increased but remained within the historic control data.

The developmental effects in the study in rats are summarized in the table below.

Table 5.9-3 Developmental effects in the rat study.

Dose (mg/kg bw/day)		0	0.4	0.8	1.6
	No. of dead fetuses/no. of fetuses studied	0/319	0/320	0/279	0/326
	Malformations -exencephaly -cleft palate			 1[b]	 1[a] 1
	Skeletal deviations -lumbar rib (no / %) -lumbar count variation (no / %) - no. of litters with fetal variations / no. of litters examined	 44 / 14 1 / 0.3 13 / 23	 41 / 13 1 / 0.3 18 / 24	 45 / 16 1 / 0.4 14 / 24	 72 / 22 5 / 1.5 16 / 24

a: conjoined twin

b: anasarca, micrognathia, cleft palate, protruding tongue, ectromelia

Based on the absence of effects in the highest dose group, the NOAEL for maternal toxicity in this study is 1.6 mg/kg bw/day.

Based on the occurrence of cleft palate, changed sex ratio and increased number of fetuses with lumbar rib and lumbar count variation in the highest dose group, the NOAEL for developmental toxicity in this study is 0.8 mg/kg bw/day.

Rabbits

In a teratogenicity study with rabbits two deaths and one premature sacrifice occurred in abamectin-treated groups. Death was preceded by reduced food and water consumption in 2 animals and by blood-stained urine in the cage of the other animal. The relationship of these deaths to treatment with abamectin is equivocal since a dose-related increase in incidence did not occur. There were no clinical signs of toxicity at any dose level.

The food and water consumption of all groups was variable, but by subjective assessment, the periods of reduced food and water consumption in the group treated at 2.0 mg/kg bw/day were more prolonged and pronounced than in the other groups. This treatment-related maternal toxicity at 2.0 mg/kg bw/day manifested as decreased food and water consumption resulted in weight loss during the dosing period which was statistically significant between day 6 and 18 of gestation compared to control. The average weight loss at 2.0 mg/kg bw/day over this period was 64 g compared to a weight increase of 64 g in the control group. The effects observed at 2.0 mg/kg bw/day are considered as evidence for maternal toxicity but not as marked maternal toxicity because the differences in maternal body weight are only small (3%) compared to the average weight of a rabbit of approximately 4 kg.

There were no treatment-related effects at any dose level on pre-implantation loss and post implantation loss, and mean foetal weight (sexes combined) at any dose level. Higher numbers of dead fetuses and an increased m/f sex ratio was observed in the group treated at 1.0 mg/kg bw/day, but not at the higher dose level. Therefore, these effects are considered incidental.

In the high dose group, the number of resorptions and the % malformed fetuses were increased. In the high dose group one litter contained 2 fetuses with cleft palate and 2 fetuses with omphalocele. In this litter 3 fetuses had sternebral malformations, including one of the fetuses with cleft palate. In 3 other litters of the high dose group in total 5 fetuses with clubbed fore-feet were found. One fetus with clubbed fore-feet also had a lumbar vertebral malformation. The incidences of these malformations are higher than the concurrent and historical control groups (not available) and were considered treatment related (by the study author).

Two fetuses in one litter from a female treated at 1.0 mg/kg bw/day also had clubbed fore-feet but the occurrence is considered not to be treatment-related because higher incidences of the defect have been recorded in historical controls (not available), one fetus from a concurrent control female also had a clubbed fore-foot, and no other malformations were observed at this dose.

The study report contains no information to relate the malformed pups with individual dams and their weight changes over pregnancy.

At 2.0 mg/kg bw/day, increased incidences of incomplete ossification of sternebrae and metacarpals are considered to reflect a treatment-related slight delay in ossification.

The developmental effects in the study in rabbits are summarized in the table below.

Table 5.9-4 Developmental effects in the rabbit study.

Dose (mg/kg bw/day)		**0**	**0.5**	**1.0**	**2.0**	**dr**
Litter response	Live fetuses	No toxicologically relevant effects				
	Fetal weight	No toxicologically relevant effects				
	Resorptions/implants (litter mean)	0.049	0.038	0.036	0.065	
	Pre implantation loss	No toxicologically relevant effects				
	Foetal implantation loss	No toxicologically relevant effects				
	Post implantation loss	No toxicologically relevant effects				
Fetus examination	No. of abnormal fetuses	No toxicologically relevant effects				
	No. of dead fetuses / no. of fetuses studied	0/97	1/91	5/100	0/121	
	Sex ratio (m:f)	1 : 0.98	1 : 1.07	1 : 1.17	1 : 1.02	
	% malformed fetuses	3.1	4.4	4.0	12.4	
	External observations and visceral deviations -cleft palate -clubbed fore-foot -omphaloceles	 0 1 1	 0 0 0	 0 2 0	 2[a] 5[a] 2[a]	
	Skeletal deviations -sternebral malformation -incompletely ossified sternebra -incompletely ossified metacarpal -incompletely ossified phalanx	 0 17 8 19	 0 17 15 27	 0 16 7 12	 3 42 33 31	

a: The 2 fetuses with cleft palate and 2 fetuses with omphaloceles were all from a single litter and 5 fetuses with clubbed fore-foot were from 3 other litters.

Summary of developmental toxicity studies

Table 5.9-5 Summary of teratogenicity studies

Species	Study type or duration; Dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effect at LOAEL	Reference
Rat	Oral developmental study (gavage); OECD 414. Day 6-19 of gestation 0, 0.25, 0.5, 1.0 and 2.0 mg/kg bw (range-finding study) 0, 0.4, 0.8 and 1.6 mg/kg bw/day (main study)	Maternal: 1.6 Developm: 0.8	>1.6 1.6	- Cleft palate, lumbar rib and lumbar count variation	Gordon, L.R.(1982a)
Rabbit	Oral developmental study (gavage); OECD 414. Day 6-27 of gestation 0, 0.5, 1.0, 2.0 and 3.0 mg/kg bw (range-finding study) 0, 0.5, 1.0 and 2.0 mg/kg bw/day (main study)	Maternal: 1.0 Developm: 1.0	2.0 2.0	food consumption and weight loss during gestation, increased number of resorptions. Developmental: cleft palate, omphalocele, clubbed fore-feet and delayed ossification	Gordon, L.R. (1982b)

The NOAEL of abamectin for maternal toxicity in rabbits in this study is 1.0 mg/kg bw/day, based on decreased water and food consumption and weight loss during gestation at 2.0 mg/kg bw/day.

The NOAEL of abamectin for foetal toxicity was also established at 1.0 mg/kg bw/day based on the occurrence of increased number of resorptions, delayed ossification and excess incidences of cleft palate, omphalocele and clubbed fore-feet at the maternally toxic dose level of 2.0 mg/kg bw/day.

5.9.3 Human data

No data available.

5.9.4 Other relevant information

None

5.9.5 Summary and discussion of reproductive toxicity

Fertility

In the 2-generation study of reproductive toxicity no effect on reproductive parameters were found.

Classification proposals according to Directive 67/548/EEC and Regulation (EC) 1272/2008

Abamectin needs not to be classified for reproductive effects according to Directive 67/548/EEC or to CLP Regulation.

Developmental toxicity

In the reproductive toxicity study no effects were observed in the pups at the time of birth. It should be noted however that this study was not designed to investigate prenatal developmental effects of abamectin.

In the developmental toxicity studies in the rat and the rabbit teratogenic effects were observed, albeit at low incidences. In the rat, developmental toxicity was observed in the absence of maternal toxicity.

Classification proposals according to Directive 67/548/EEC

In view of the low incidences of the developmental effects it is considered that there is some but not clear evidence of a developmental effect in rats. In the developmental toxicity study in the rat there is actually only 1 cleft palate to take into consideration for classification and labelling. As stand alone study, this would not be considered relevant for classification and labelling, but in combination with the rabbit study, this one cleft palate should be considered.

In the rabbit study there is one ‘strange’ litter with 2 fetuses with cleft palate and 2 fetuses with omphaloceles. The relevance of these findings for classification and labelling can be questioned. The increase in malformations (clubbed fore-foot) in rabbits at the highest dose is above the concurrent and historic controls and therefore treatment related. The increased incidence of these malformations was small (5 cases in the high dose versus 1 in the controls) and therefore considered as evidence but no clear evidence. This effect was observed in presence of slight maternal toxicity including weight loss (3%) and decreased water and food consumption. The decrease in body weight is considered small in relation to the average weight of a rabbit and is not considered as

marked maternal toxicity. Also, it is considered unlikely that the increased incidence in malformation is caused by the reduced body weight. The increase in club fore-foot is considered to be a direct effect of the substance and not a secondary consequence of the maternal toxicity. As the time of development of this effect is unknown, it is unknown whether the differences in p-glycoprotein development between rats, rabbits, and humans is also important for this effect. Therefore, it is assumed that this effect is also relevant to humans. It is proposed to classify abamectin for harm to the unborn child as Repro Cat. 3; R63 based on an increased incidence (but no clear increase) in malformations (clubbed fore-foot) which is considered not secondary to maternal toxicity and relevant to humans.

Classification proposals according to Regulation (EC) 1272/2008

The same argumentation as provided above for classification according to Directive 67/548/EEG applies also for classification according to Regulation (EC) 1272/2008. It is proposed to classify abamectin with Repr. Cat. 2, H361d.

Effects on or via lactation

The increase in post-natal mortality in the 2-generation study in rats at 0.4 mg/kg bw/day is most likely an effect on or via lactation. This is confirmed by a cross fostering study with the closely related substances ivermectine, which indicates that the neonatal toxicity was primarily a function of postnatal exposure ((Merck & Co., Inc., 1980f as summarised by JECFA, 1991). Therefore, these effects would be considered relevant for effects on or via lactation and not for developmental effects. Significant neonatal mortality seen in rat pups is likely to be the result of a lack of p-glycoprotein expression in the neonatal rat brain. P-glycoprotein dependent xenobiotic efflux in the blood brain barrier is considered to play an important role in attenuating neurotoxicity of avermectins. However, brain p-glycoprotein expression starts early in human development, having been detected in human foetal brain microvessels as early as week eight of pregnancy. Expression of p-glycoprotein in the cerebrum and cerebellum of rats is not fully developed in neonate rats and expression in the jejunal epithelial brush borders does not start before post-natal day 8. Adult levels are reached at post-partum day 20 or 28. Therefore, the increased postnatal mortality is considered not to be relevant to human risk assessment. For further information on the role of p-glycoproteins in abamectin toxicity see section 5.10.1.

Classification proposals according to Directive 67/548/EEC

No classification for effects on or via lacatation is proposed because the increased post natal toxicity observed in the 2-generation study in rats is not considered relevant to humans.

Classification proposals according to Regulation EC 1272/2008

No classification for effects on or via lacatation is proposed because the increased post natal toxicity observed in the 2-generation study in rats is not considered relevant to humans.

5.10 Other effects

5.10.1 P-glycoprotein expression and increased susceptibility to abamectin

From the studies submitted by the registrant, it was concluded that CF-1 mice exhibit typical clinical signs of neurotoxicity and have an increased susceptibility to abamectin toxicity. From those studies it was suggested that the increased susceptibility of CF-1 mice (compared to CD-1

mice) is related to the accessibility of the 8,9-Z isomer to the target organ, and hence to the presence or absence of p-glycoprotein expression. In order to investigate this suggestion, several studies were performed to investigate the relation between p-glycoprotein and the increased sensitivity of CF-1 mice to abamectin and the 8,9-Z isomer.

Comparative studies of the acute oral toxicity of abamectin in pregnant and non-pregnant CF-1 mice and a maternal toxicity study by dietary administration during gestation have been performed. The influence of the mdr1 genotype and p-glycoprotein levels on the expression of abamectin toxicity were investigated in two exploratory studies in CF-1 mice of known genotype and in CF-1 and CD-1 strain mice of unknown genotype. A summary of these studies is presented in the table below.

Table 5.10-1 Summary of supplementary studies

Study/ species dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOEL	Reference
10-day dietary maternal toxicity; CF-1 mice;	- maternal: 0.08 (time-weighted)	- maternal: 0.24 (time-weighted)	Tremors, hunched posture, poor condition	Gordon, L.R. (1984g)
Acute oral toxicity; Pregnant / non-pregnant CF-1 mice	LD_{50} non-pregnant mice: >20 and <40 mg/kg bw LD_{50} pregnant mice: 19 mg/kg bw	< 5 5	Deaths, tremors, bradypnea Deaths, tremors, bradypnea	Gordon, L.R. (1986h)
Acute oral toxicity; Pregnant / non-pregnant CF-1 mice	LD_{50} non-pregnant mice: 15.0 mg/kg bw LD_{50} pregnant mice: 11.8 mg/kg bw	< 5 < 5	Death, loss of righting reflex, bradypnea Death, tremors, bradypnea, clonic convulsions	Gordon, L.R. (1986h)
Exploratory acute oral toxicity; CF-1 mice of known genotype for p-glycoprotein	LD_{50} (+/+ genotype female mice): 28 mg/kg bw LD_{50} (+/- genotype female mice): 14 mg/kg bw	< 10 < 10	Tremors, bradypnea, decreased activity. Tremors, bradypnea, decreased activity, weight loss during first week	Hall, S. (1997)
Exploratory oral toxicity; CF-1 / CD-1 mice (dose = 0.8 mg/kg bw for 4 days)	Results: All CF-1 mice showed tremors and ataxia, but 17% also showed dyspnea, lateral recumbence and coma (= sensitive to abamectin toxicity). All but one sensitive animal had no detectable p-glycoprotein in brain and small intestine. All insensitive CF-1 mice evaluated and all CD-1 mice had detectable p-glycoprotein levels. Control and treated CD-1 mice had similar levels of p-glycoprotein.			Lankas, G.R. (1994)

Exploratory study of p-glycoprotein development in rat fetuses and pups.	Results: the expression of p-glycoprotein in the cerebrum and cerebellum is not fully developed in neonate rats. P-glycoprotein expression reaches adult levels by post-natal day 20. Expression of p-glycoprotein in the jejunal epithelial brush border does not start before post-natal day 8. It is suggested that neonate rats with limited or no p-glycoprotein expression have an increased susceptibility to avermectin toxicity.	Cukierski, M.A. (1995), Lankas, G.R. (1996b, addendum)
Examination of developmental expression of p-glycoprotein levels in rat pups Rats postnatal days 1,3,7,14,21,28,56,84 examined	Results: p-glycoprotein was first detected at post-natal day 7 in pups, with subsequent increases to plateau at adult levels by post-natal day 28. In the adult rat brain, p-glycoprotein was detected predominantly in the membrane fraction. Double immunostaining of p-glycoprotein and von Willebrand factor demonstrated that p-glycoprotein was co-localised with brain capilliaries, suggesting a role for p-glycoprotein in the blood brain barrier.	Matsuoka, Y. et al. (1999)

Dietary administration of abamectin to pregnant CF-1 mice during organogenesis resulted in clinical signs of neurotoxicity at time-weighted average maternal dose levels above 0.08 mg/kg bw/day, whereas no treatment-related effects were observed on reproductive parameters.

In two studies with pregnant and non-pregnant CF-1 mice, singly orally exposed to abamectin at day 10, 11 or 12 of gestation, it was shown that the LD_{50}'s in pregnant animals were slightly, not statistically significantly lower (LD_{50} = 19 mg/kg bw and LD_{50} = 11.8 mg/kg bw in study 1 and 2, respectively) compared to the LD_{50}'s in non-pregnant mice (LD_{50} = between 20 and 40 mg/kg bw and LD_{50} = 15 mg/kg bw in study 1 and 2, respectively). Typical clinical signs of neurotoxicity (tremors, clonic convulsion and bradypnea) occured in both pregnant and non-pregnant animals.

In a study with female CF-1 strain mice, heterozygous (+/-) or homozygous positive (+/+) for the mdr1 gene (which codes for p-glycoprotein expression), the LD_{50} for abamectin in homozygous positive (+/+) female mice was 28 mg/kg bw, whereas the LD_{50} in heterozygous female mice was 14 mg/kg bw.

In a comparative study with CF-1 mice and CD-1 mice, it was demonstrated that 17% of a random population of CF-1 mice are sensitive to abamectin toxicity, showing signs of neurotoxicity (tremors, ataxia, dyspnea, lateral recumbency, coma) in response to 0.8 mg/kg bw/day abamectin for 4 days. Sensitive CF-1 individuals were shown to express no p-glycoprotein in the cerebrum, cerebellum and jejunum, whereas "non-sensitive" CF-1 mice and all CD-1 mice were shown to express p-glycoprotein in these tissues. Control and treated CD-1 mice had similar levels of p-glycoprotein.

Role of p-glycoprotein in limiting avermectin toxicity

C57BL/6 derived abcb1a knockout mice and some CF-1 mice were found to exhibit ivermectin sensitivity. CF-1 mouse ivermectin sensitivity exhibited classic Mendelian inheritance patterns, and has since been shown to be due to retroviral insert in exon 23 of the abcb1a gene in some CF-1 mice. This results in total absence of properly transcribed, functional pgp in CF-1 mice homozygous for the disrupted form of the gene.

In both the CF-1 and the C57BL/6 mdr1a null mice models oral ivermectin dosing results in plasma ivermectin concentrations 2.5-fold to 3.3-fold higher in pgp null mice than in the wild type mice 24 h after dosing. Lack of pgp dependent efflux at the Blood Brain Barrier (BBB) also allows vastly increased brain penetration of avermectins. Brain ivermectin concentrations 24 h post dosing are between 33-fold and 87-fold higher in pgp null mice compared to wild type mice. Studies in our

laboratory have shown similar results for two other avermectins, emamectin and abamectin, which are used predominantly as pesticides. Homozygous pgp null (abcb1a –/–) mice show increased susceptibility to 0.2 mg/kg oral abamectin, while heterozygous (abcb1a +/–) mice and wild type mice (abcb1a +/+) are insensitive to up to 2.5 mg/kg abamectin. LD_{50} data indicates that at very high doses heterozygous mice are slightly more abamectin sensitive than homozygous wild type mice (–/+ LD_{50} = 14 mg/kg, +/+ LD_{50} = 30 mg/kg, –/– LD_{50} = 0.3 mg/kg). Thus although heterozygous mice express less brain pgp, a single copy of a functional abcb1a gene is sufficient for adequate pgp functionality in the mouse BBB at doses of avermectins used in the clinic (0.2 mg/kg), or resulting from worker pesticide exposure.

Where placental pgp activity is compromised avermectins can also exhibit developmental toxicity. In pgp null mice foetal avermectin exposure is associated with increased incidence of cleft palate. The placenta is a foetal tissue, and as such avermectin developmental toxicity is dependent on the abcb1a status of the fetus. CF-1 abcb1a –/– fetuses of mothers treated with abamectin have significantly higher concentrations of abamectin in their plasma than their abcb1a +/+ and +/– littermates. Similarly when CF-1 dams were dosed with 1.5 mg/kg abamectin, all abab1a –/– fetuses developed cleft palates, while none of their abcb1a +/+ littermates and only 30% of their +/– littermates developed cleft palates.

Significant neonatal ivermectin neurotoxicity is seen in rat pups through a combination of ivermectin exposure of the offspring of ivermectin dosed rat dams via the dams' milk, and lack of pgp expression in the neonatal rat brain. However, this is not thought to be relevant to human risk assessment as brain pgp expression starts early in human development, having been detected in human foetal brain microvessels as early as week eight of pregnancy.

P-glycoprotein human polymorphisms and pgp haplotypes

Naturally occurring mutations that lead to non-functional pgp have been found in both the CF-1 strain of mice and dog breeds closely related to the collie. Millions of humans have received ivermectin as an anthelminthic treatment for river blindness without reports of major adverse neurological effects, although arguably adverse effect reporting may be less robust in the areas of the world where river blindness occurs. In addition, cumulatively more than 4,000 human volunteers have been genotyped for ABCB1 [although often only for known single nucleotide polymorphisms (SNPs)] without reports of major rearrangements of the ABCB1 gene similar to those in the CF-1 mouse and collie dog. Taken together this may indicate that individuals with significantly compromised pgp functionality analogous to that seen in the CF-1 pgp –/– mouse are rare.

More than 50 naturally occurring SNPs have been identifed in the human ABCB1 gene. The vast majority are silent, i.e. they either do not occur in the coding region of the gene, or due to the inherent redundancy of codon usage they do not alter the amino acid sequence of the protein. As has been extensively reviewed elsewhere there are numerous conflicting reports of the effects of individual ABCB1 SNPs on pgp expression and function in various tissues. Also the submitted publication gives an extensive overview of publications on the effects of individual ABCB1 SNPs. The conclusion is that there is no clear pattern of clinical effect of individual SNPs on pgp mediated efflux.

It is therefore suggested that combinations of human SNPs (haplotype) may be important in determining phenotype. An overview of the literature on pgp haplotypes is presented in the publication. This includes studies in which human BBB pgp function has been measured directly. Although various human ABCB1 haplotypes and/or SNPs have been reported to alter pgp function

in relation to gut absorption, at present there is no conclusive data indicating that any of the known common haplotypes, including homozygosity for the most common minority haplotype, result in a significant loss of BBB pgp functionality. This would tend to indicate that the CF-1 mdr1a –/– mouse strain, which completely lacks pgp BBB functionality, is not a representative model for assessing risk in humans homozygous for any of the known haplotypes.

Population distribution of pgp haplotypes

Populations with different ethnicities are known to have different distributions of the various pgp haplotypes. Forty-eight and 79% of ABCB1 haplotypes found in the African American and Caucasian populations, respectively, produce a pgp identical to the reference amino acid sequence. Of the remainder, 38% of African American and 7.5% of Caucasian ABCB1 genes represent a haplotype which contains only one nonsynonymous SNP. Data from in vivo studies indicates that alleles in these two categories both produce pgp that is functional in the BBB. Given the sampled population frequencies of the commonest pgp haplotypes, and the fact that at clinically relevant doses a single functional copy of abcb1a is sufficient to prevent avermectin neurotoxicity in the CF-1 mouse, it is possible to calculate the proportion of the human populations that are likely to exhibit normal pgp BBB functionality (see publication for more details). >98% of people in African American and Caucasian populations will carry at least one copy of an ABCB1 haplotype that is already known to encode a pgp that is functional in the BBB and will therefore not be at risk of toxicity from the concentrations of avermectins to which humans are typically exposed. Between 1 and 2% of the population would thus carry only haplotypes with unconfirmed BBB functionality. Each individual "unconfirmed BBB functionality" haplotype is relatively rare within the population, often only having been identified in a single heterozygous individual, with each "rare" haplotype having an allelic frequency of less than 1%. As such, individuals that are homozygous for any one of the haplotypes with unconfirmed BBB functionality would be very rare within the population (<0.01%). If any of these rare haplotypes exhibited significantly compromised BBB pgp functionality it is likely that individuals homozygous for that haplotype, and thus having compromised BBB pgp function, would be extremely rare.

Conclusions by MacDonald & Gledhill

Pgp 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. There is currently no evidence for the existence of mutations of the ABCB1 gene in the human population that result in a loss of function analogous to that seen in the CF-1 mouse and collie dog. Although there are numerous reports for and against the proposition that some ABCB1 SNPs and/or haplotypes exhibit reduced pgp expression and function, there are no consistent data indicating that known SNPs or haplotypes have an adverse effect on pgp function in the BBB or placenta. Where human BBB pgp function has been measured directly the most common haplotypes were found to have equal functionality. Since heterozygous pgp +/– mice and dogs do not exhibit ivermectin neurotoxicity at clinically relevant doses it is likely that humans carrying at least one functional copy of ABCB1 will not be more susceptible to avermectin toxicity at clinically relevant doses or at the low exposure levels resulting from pesticide use. Calculations using allelic frequencies of known haplotypes indicate that homozygosity for any as yet uncharacterised haplotypes with severely reduced BBB functionality is likely to be very rare in human populations.

Concluding remarks

Based on the recent publication which reviews the relevant literature on p-glycoprotein polymorphism, showing that non-functional p-glycoprotein has not been identified in humans, and the supplementary studies in the DAR which show that only the –/– CF-1 mouse is more sensitive to abamectin toxicity, it can be concluded that the studies with the unique polymorphic CF-1 mouse are not relevant for human risk assessment.

Also the JMPR meeting (JMPR, 1997) concluded that the CF-1 strain mouse is not appropriate for establishing an ADI for abamectin. The EU Committee for Veterinary Medicinal Products (CVMP) reached the same conclusion in 2002 upon reviewing the abamectin toxicity data and the JMPR position.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of report.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

Abamectin has no explosive properties. No classification is required.

6.2 Flammability

Abamectin is considered not highly flammable. No classification is required.

6.3 Oxidising potential

Abamectin has no oxidising properties. No classification is required.

7 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard properties assessment for abamectin is based on the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands) and the Competent Authority Report (CAR; July 2008; RMS The Netherlands) on the inclusion of abamectin in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

All tables in the present assessment are copied from the DAR or CAR. The tables are renumbered in accordance with the paragraph numbers in chapter 7. Only data that were considered acceptable are presented.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short- and long-term toxicity to fresh water fish

The acute and chronic toxicity of abamectin and avermectin B_{1a} to fresh water fish is summarised in the table below. Note that effect concentrations are given in µg/L. The toxicity of avermectin B_{1a} to fish is similar to that of abamectin.

Table 7.1-1 Acute and chronic toxicity of abamectin and avermectin B_{1a} to fresh water fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]		Remarks	Reference
			design	duration	L/EC_{50}	NOEC		
EPA 1975	*Oncorhynchus mykiss*	LC_{50} mortality	static	96 h	3.6		abamectin; nominal	LeBlanc & Sousa, 1981
OECD 203	*Cyprinus carpio*	LC_{50} mortality	flow-through	96 h	42		abamectin; nominal	Douglas & Pell, 1985
OECD 203	*Ictalurus punctatus*	LC_{50} mortality	static	96 h	24		abamectin; nominal	McAllister et al., 1985
OECD 203	*Pimephales promelas*	LC_{50} mortality	flow-through	96 h	14.7		abamectin; actual	Bätscher, 2003
EPA 1975	*Lepomis macrochirus*	LC_{50} mortality	flow-through	96 h	7.2		avermectin B_{1a}; nominal	Forbis, 1983
OECD 204; OECD 215 (draft)	*Cyprinus carpio*	NOEC mortality; weight	flow-through	28 d		6.1	abamectin; actual	Rufli, 2000
ASTM 1983	*Oncorhynchus mykiss*	NOEC Early Life Stage	flow-through	72 d		0.52	abamectin; actual	McAllister, 1986

a: modified exposure: gradually diminishing concentrations

The acute toxicity of the major degradation products: 8a-hydroxy-avermectin B_{1a}. and [8,9-Z]-avermectin B_{1a} is summarised in the table below. The data shows that these degradation products are not more toxic than the parent compound.

Table 7.1-2 Acute toxicity of 8a-hydroxy-avermectin B_{1a}. and [8,9-Z]-avermectin B_{1a} to fresh water fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]	Remarks	Reference
			design	duration	L/EC_{50}		
OECD 203	*Oncorhynchus mykiss*	LC_{50} mortality	semi-static	96 h	520 µg/L	8a-hydroxy-avermectin B_{1a} (NOA 448112), measured	Peither, A. (2001a)

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]	Remarks	Reference
			design	duration	L/EC_{50}		
OECD 203	*Oncorhynchus mykiss*	LC_{50} mortality	flow-through	96 h	5.1 µg/L	[8,9-Z]-avermectin B_{1a} (NOA 427011)	Sutherland, C.A., Kendall, T.Z, Krueger, H.O (2000a)

Short- and long-term toxicity to marine fish

Accepted data for marine fish are summarised in Table 7.1-3.

Table 7.1-3 Acute toxicity to saltwater fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]		Remarks	Reference
			design	duration	L/EC_{50}	NOEC		
ASTM 1982	*Cyprinodon variegatus*	LC_{50} mortality	static	96 h	15		abamectin; nominal	Ward, 1985

7.1.1.2 Aquatic invertebrates

Short- and long-term toxicity to fresh water invertebrates

The acute and chronic toxicity of abamectin and avermectin B_{1a} to freshwater invertebrates is summarised in the table below. Note that effect concentrations are given in µg/L. The toxicity of avermectin B_{1a} to daphnids is similar to that of abamectin.

Table 7.1-4 Acute and chronic toxicity of abamectin and avermectin B_{1a} to invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]		Remarks	Reference
			design	duration	L/EC_{50}	NOEC		
EPA 1975	*Daphnia magna*	LC_{50} mortality	static	48 h	0.34		abamectin; nominal	LeBlanc & Surprenant, 1981
EPA	*Daphnia magna*	EC_{50} immobilisation	static	48 h	0.37		abamectin; actual	Forbis, 1989a
EPA	*Daphnia magna*	EC_{50} immobilisation	static; sediment spiked	48 h	0.26		abamectin; actual water	Forbis, 1989b
EPA; OECD 202	*Daphnia magna*	EC_{50} immobilisation	static	48 h	0.56		abamectin; actual initial	Rufli, 1998
EPA	*Daphnia magna*	EC_{50} immobilisation	static	48 h	0.3		abamectin; nominal	Naimie et al., 1985
EPA	*Daphnia magna*	EC_{50} immobilisation	static	48 h	0.63		avermectin B_{1a}; nominal	Naimie et al., 1985
EPA; OECD 202	*Daphnia longispina*	EC_{50} immobilisation	static	48 h	0.38		abamectin; actual	Knauer, 2001b
EPA; OECD 202	*Daphnia pulex*	EC_{50} immobilisation	static	48 h	0.12		abamectin; actual	Knauer, 2001b
EPA; OECD 202	*Daphnia pulex*	EC_{50} immobilisation	static	48 h	0.28		abamectin; nominal	Knauer, 2001c
EPA; OECD 202	*Daphnia galeata*	EC_{50} immobilisation	static	48 h	0.55		abamectin; nominal	Knauer, 2001a
EPA; OECD 202	*Simocephalus sp.*	EC_{50} immobilisation	static	48 h	0.30		abamectin; actual	Knauer, 2001b
EPA; OECD 202	*Diaphanosoma sp.*	EC_{50} immobilisation	static	48 h	0.53		abamectin; nominal	Knauer, 2001c
EPA; OECD 202	*Thamnocephalus platyurus*	EC_{50} immobilisation	static	48 h	30		abamectin; nominal	Knauer, 2001d
EPA; OECD 202	*Thamnocephalus platyurus*	EC_{50} immobilisation	static	48 h	2.8		abamectin; actual	Knauer, 2001e
EPA; OECD 202	*Brachionus calyciflorus*	EC_{50} immobilisation	static	48 h	4000		abamectin; actual	Knauer, 2001e
EPA; OECD 202	*Chaoborus sp.*	EC_{50} immobilisation	static	48 h	190		abamectin; actual	Knauer, 2001f
EPA; OECD 202	*Chaoborus sp.*	EC_{50} immobilisation	static	48 h	41		abamectin; nominal	Knauer, 2001g
EPA; OECD 202	*Cloeon sp.*	EC_{50} immobilisation	static	48 h	2.9		abamectin; nominal	Knauer, 2001g

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]		Remarks	Reference
			design	duration	L/EC_{50}	NOEC		
EPA; OECD 202	*Gammarus sp.*	EC_{50} immobilisation	static	48 h	6.2		abamectin; nominal	Knauer, 2001h
EPA; OECD 202	*Gammarus sp.*	EC_{50} immobilisation	static	48 h	8.6		abamectin; actual	Knauer, 2001i
EPA; OECD 202	*Lymnea stagnalis*	EC_{50} immobilisation	static	48 h	55		abamectin; actual	Knauer, 2001j
OECD 211	*Daphnia magna*	NOEC mortality	semi-static	21 d		0.010	abamectin; nominal	Pfeifle, 2001a
OECD 211	*Daphnia magna*	NOEC mortality	flow-through	21 d		0.030	avermectin B_{1a}; actual	Surprenant & Mastone, 1983

Short- and long-term toxicity to salt water invertebrates

Accepted data for marine invertebrates are summarized in Table 7.1-5. 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.

The difference between the static LC_{50} of 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 of abamectin.

Table 7.1-5 Acute and chronic toxicity to saltwater organisms

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]		Remarks	Reference
			design	duration	L/EC_{50}	NOEC		
EPA 1970, APHA 1980	*Mysidopsis Bahia*	LC_{50} mortality	static	96 h	0.21		abamectin ; nominal	Forbis & Burgess, 1985
EPA 1970, APHA 1980	*Mysidopsis bahia*	LC_{50} mortality	flow-through	96 h	0.020*		abamectin ; actual	Suprenant, 1988
BMRL	*Crassostrea virginica*	EC_{50} larval development	static	48 h	430		abamectin ; nominal	Ward, 1983
BMRL	*Penaeus duorarum*	LC_{50} mortality	static	96 h	1.6		abamectin ; nominal	Ward, 1983
BMRL	*Callinectus spidus*	LC_{50} mortality	static	96 h	153		abamectin ; nominal	Ward, 1983
	Mysidopsis bahia	NOEC reproduction	flow-through	28 d		0.0035	abamectin ; actual	Suprenant, 1988

*) the toxicity at different ages were tested (≤1 to 21 days old mysids). LC_{50} tends to increase with increasing age (from 0.020 to 0.026 µg/L), the lowest value is used for classification of abamectin. .

Table 7.1-6 Acute toxicity to invertebrates of metabolites of abamectin and avermectin B_{1a}

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg/L]	Remarks	Reference
			design	duration	L/EC_{50} [µg/L]		
US EPA 1975 and 1983	*Daphnia magna*	LC_{50} mortality	static	48 h	>100	polar photolysis products of avermectin B_{1a}	Naimie, H., Anton, S. and Kaelin L. (1985)
US EPA 1975 and 1983	*Daphnia magna*	EC_{50} immobilisation	static	48 h	6.8	moderately polar photolysis product of avermectin B_{1a}	

Guideline / Test method	Species	Endpoint / Type of test	Exposure			Results [µg/L]	Remarks	Reference
			design	duration		L/EC_{50} [µg/L]		
US EPA 1975 and 1983	*Daphnia magna*	EC_{50} immobilisation	static	48 h		27.2	non-polar photolysis product of avermectin B_{1a}, 52 % [8,9-Z]-avermectin B_{1a} and 62 % avermectin B_{1a}	
US EPA 1975 and 1983	*Daphnia magna*	EC_{50} immobilisation	static	48 h		26	8a-hydroxy-avermectin B_{1a} (NOA 448112), nominal	Forbis, A.D., Georgie, L. and Burgess, D. (1985)
OECD 202 US EPA FIFRA 72-2	*Daphnia magna*	EC_{50} immobilisation	static	48 h		1.6	8a-hydroxy-avermectin B_{1a} (NOA 448112), measured	Peither, A. (2001)
US EPA 1975 APHA 1980	*Daphnia magna*	EC_{50} immobilisation	static	48 h		14	[8,9-Z]-avermectin B_{1a} (NOA 427011), nominal	Forbis, A.D., Georgie, L. and Burgess, D. (1985)
OECD 202 US EPA FIFRA 72-2	*Daphnia magna*	EC_{50} immobilisation	static	48 h		0.082	[8,9-Z]-avermectin B_{1a} (NOA 427011), measured	Peither, A. (2001)
OECD 202	*Daphnia magna*	EC_{50} immobilisation	static	48 h		0.28	4"-oxo-avermectin B_{1a} (NOA 426289) nominal	Bätscher, R. (2003b)

7.1.1.3 Algae and aquatic plants

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. These same hold true for the degradation products as shown in the table below.

Table 7.1-7 Toxicity to aquatic plants (algae)

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results	Remarks	Reference
			design	duration	NOEC		
OECD 201	*Pseudokirchneriella subcapitata*	E_rC_{50} growth inhibition	static	72 h	> 6.1 mg/L	8a-hydroxy-avermectin B_{1a} measured	Peither, A. (2001)
OECD 201	*Pseudokirchneriella subcapitata*	E_rC_{50} growth inhibition	static	72 h	> 10 mg/L	[8,9-Z]-avermectin B_{1a} (NOA 427011), nominal initial	Sutherland, C.A., Kendall, T.Z. and Krueger, H.O. (2000)

7.1.1.4 Sediment organisms

The toxicity of abamectin to *Chironomus riparius* is summarised in table 7.1-8.

Table 7.1-8 Chronic toxicity to sediment organisms

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results	Remarks	Reference
			design	duration	NOEC		
draft BBA draft OECD	*Chironomus riparius*	NOEC emergence	static; sediment	28 d	3.3 µg/kg dw	avermectin B_{1a}; nominal initial	Grade, 2002

7.1.1.5 Other aquatic organisms

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of report.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro organisms

Not applicable for this type of report.

7.2.1.2 Toxicity to terrestrial plants

Not applicable for this type of report.

7.2.1.3 Toxicity to soil micro-organisms

Not applicable for this type of report.

7.2.1.4 Toxicity to other terrestrial organisms

Not applicable for this type of report.

7.2.2 Calculation of Predicted No Effect Concentration (PNEC soil)

Not relevant for this type of report.

7.3 Atmospheric compartment

No data available

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

Not applicable for this type of report.

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of report.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of report.

7.6 Conclusion on the environmental classification and labelling

Abamectin is hydrolytically stable. Under both natural and artificial light condition the half-live of abamectin in water is between 1 and 2 days. Abamectin is not readily biodegradable as only 3% degradation was observed in an OECD301F test after 28 days. Abamectin is not readily degradable as the $DT_{50,system}$ obtained in aerobic and anaerobic simulation studies in water/sediment systems was 87-91 days and 230-312 days, respectively. The DT50 obtained from the water/sediment system is considered most relevant and is therefore used for the classification of abamectin.

Abamectin has a log Kow of 4.4. In a BCF study, a BCF value of 69 was obtained based on plateau total radioactive residue in whole fish and average total radioactive residue in water, whereas a BCF value of 52 was obtained based on uptake and elimination rate constants. The measured BCF value is considered reliable and therefore used for the classification of abamectin.

Abamectin generally produces LC_{50} and EC_{50} values in the µg/l range in fish and crustaceans. The lowest LC_{50} value obtained for abamectin in freshwater fish, freshwater crustaceans and marine crustaceans is 3.6 µg/l, 0.12 µg/l and 0.020 µg/l, respectively. The LC_{50} value of 0.020 µg/l is used for the classification of abamectin.

The toxicity of the major degradation products was not higher than that of the parent compound.

Conclusion of environmental classification according to Directive 67/548/EEC

In acute aquatic toxicity studies, L(E)C50 values in fish and crustaceans were obtained at abamectin concentrations <1 mg/l. Abamectin is not readily biodegradable. Abamectin therefore fulfils the criteria for classification with N; R50/53.

Given the very low acute toxicity to invertebrates, in accordance with Directive 67/548/EEC and Regulation (EC) 1272/2008, the following Specific Concentration Limits should apply:

Classification of the preparation/mixture		
N; R50-53 H400, H410	N; R51-53 H411	R52-53 H412
Cn ≥ 0.0025%	0.00025% ≤ Cn <0.0025%	0.000025% ≤ Cn <0.00025%

where Cn is the concentration of abamectin/avermectin B_{1a} in the preparation/mixture.

Conclusion of environmental classification according to Regulation (EC) 1272/2008

In acute aquatic toxicity studies, L(E)C50 values in fish and crustaceans were obtained at abamectin concentrations <1 mg/l. Abamectin is not rapidly degradable based on 3% degradation in a ready biodegradability study, and a $DT_{50,system}$ of 87-91 days and 230-312 days in aerobic and anaerobic water/sediment simulation studies. Abamectin therefore fulfills the criteria for classification as aquatic environmental hazard acute category 1, H400 and aquatic environmental hazard chronic category 1, H410. The signal word is 'Warning' and the environmental hazard pictogram is required.

The M-factor for abamectin is 10,000. This value is based on two LC_{50} values of 0.020 µg/l and 0.022 µg/l obtained for the marine crustacean *Mysidopsis bahia* in a 96-h flow-through study.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Harmonised classification is required because this substance is an active substance in the meaning of Directive 91/414/EEC and Directive 98/8/EC (Regulation (EC) 1272/2008, Article 38 (1a)).

REFERENCES

The classification and labeling proposal is based on the data provided in:

- the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of abamectin in Annex I of Council Directive 91/414/EEC (DAR October 2005 + addendum February 2008, RMS The Netherlands)
- the Competent Authority Report (CAR; July 2008; RMS The Netherlands) on the inclusion of abamectin in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.
- JECFA, 1991, Toxicological evaluation of certain veterinary drug residues in food, IPCS, WHO Food additive series 27, Geneva, 1991.