Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Emamectin benzoate (ISO); (4’’R)-4’’-deoxy-4’’-(methylamino)avermectin B1 benzoate

EC Number: -

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

Index Number:-

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1 PHYSICAL HAZARDS

1.1 Explosives

Study reference: Angly, 2000c, study report MK244/0221

Test type: steel sleeve test, according to Directive 92/69/EEC, Part A.14

Detailed study summary and results:

Material and methods

The test item was tested as delivered.

Results

Test no.	Nozzle-plate with 6 mm hole		Nozzle plate with 2 mm hole	
	explosion	Number of fragments	explosion	Number of fragments
1	no	-	no	-
2	no	-	no	-
3	no	-	no	-

The test item is not thermally sensitive.

1.2 Flammable gases (including chemically unstable gases)

Not applicable (emamectin is not a gas).

1.3 Oxidising gases

Not applicable (emamectin is not a gas).

1.4 Gases under pressure

Not applicable (emamectin is not a gas).

1.5 Flammable liquid

Not applicable (emamectin is not a liquid).

1.6 Flammable solids

Study reference: Angly, 2000a (MK244/0220)

Test type:

According to Directive 92/69/EEC, Part A.10. The study is performed according to GLP.

Detailed study summary and results:

Material and methods

The test item was tested as delivered, *i.e.* not dried and not sieved. Moisture contents: 0.2% by drying (24h, 60°C, <100 mbar).

Results

Preliminary test

Burning time to 200 mm: -

Smouldring fire does not spread out.

Main test

Test No.	Burning time to 100 mm (s)	Remarks
1	-	Has not to be carried out! (according to EEC A.10)
2	-	
3	-	
4	-	
5	-	
6	-	

Not highly flammable.

1.7 Self-reactive substances

Data lacking.

1.8 Pyrophoric liquids

Not applicable (emamectin is not a liquid).

1.9 Pyrophoric solid

Data lacking.

1.10 Self-heating substances

Study reference: Angly H., 2000b (MK244/0222)

Test type

EEC A.16

Detailed study summary and results:

Information from abstract only:

- Method: Measured (EEC A5; OECD 115 Wilhelmy plate method)
- Result: Surface tension at 20 °C (96.5%): 48.8 mN/m (90 % saturated solution)

1.11 Substances which in contact with water emit flammable gases

Data lacking.

1.12 Oxidising liquids

Not applicable (emamectin is not a liquid).

1.13 Oxidising solids

Study reference: Angly, 2000 (MK244/0223)

Test type

Directive 92/69/EEC, Part A.17

The study is performed according to GLP.

Detailed study summary and results:

Material and methods

Ignition source: hot flame from a gas burner.

The combustible substance (cellulose powder with fibre-lengths of more than 85% between 0.020 and 0.075 mm, particle size 99.6% <0.125 mm derived by sieve-analysis) was dried at 105°C until constant weight was obtained.

The test item was tested as delivered (particle size 99.0% <0.125 mm determined by sieve-analysis) and was dried at 105°C until constant weight was obtained.

Results

Max. burning rate of the reference mixture barium nitrate/ cellulose 60:40 = 2.94 mm/s.

Initial test

Mixture (%)		(1) Burning rate (mm/s)	Reaction
Test item	cellulose		
20	80	2.60	Steady burning with flame throughout the whole mass.
40	60	2.22	Steady burning with flame throughout the whole mass.
60	40	1.87	Steady burning with flame throughout the whole mass.
80	20	0.87	Steady burning with flame throughout the whole mass.

(1) Only one test

Main test

Mixture (%)		(2) Burning rate (mm/s)	Reaction
Test item	cellulose		
5	95	2.50	Steady burning with flame throughout the whole mass.
20	80	2.63	Steady burning with flame throughout the whole mass.
30	70	2.38	Steady burning with flame throughout the whole mass.

(2) In a run of six tests

(3) Max. burning rate: mixture with 80% cellulose and 20% of test item.

$$\frac{(3)\,\text{max. burning rate test mixture}}{(4)\,\text{max. burning rate reference mixture}} = \frac{2.63\ \text{mm/s}}{2.94\ \text{mm/s}} = 0.89$$

1.14 Organic peroxides

No data available.

1.15 Corrosive to metals

No data available.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 STUDY 1 – Absorption, distribution, metabolism and excretion

Characteristics

Type of study	:	Absorption, distribution, metabolism and excretion	Exposure	:	Single or repeated by gavage
Year of execution	:	1994-1995	Doses	:	0.5 mg/kg bw/day (single and repeated exposure) and 20 mg/kg bw (single exposure)
Test substance	:	MK-0244 (purity 95.4%, with 89.1%[a] MAB1a and 6.3% MAB1b (^{3}H)-MAB1a: 4"-deoxy-4"-epimethylaminoavermectin B1a benzoate (radiochemical purity 97.63% and 98.24%; spec. act. 16.020 mCi/mg and 15.258 mCi/mg)	Vehicle	:	Propylene glycol/saline (50:50 v/v)
Route	:	oral	GLP statement	:	yes
Species	:	Rat, Crl:CD(SD)BR	Guideline	:	-
Group size	:	4/sex/dose (biliary excretion after single exposure) 6/sex/dose (pharmacokinetics) 12/sex/dose (tissue distribution/ excretion)	Acceptability	:	acceptable

a: is lower than the by the notifier stated composition of emamectin with " at least 90% MAB1a".

Study design

Rats were treated orally, by gavage, with emamectin (MK-0244) and/or (^{3}H)-MAB1a (4"-deoxy-4"-epimethylaminoavermectin B1a benzoate) in propylene glycol/saline. (^{3}H)-MAB1a was administered orally, by gavage, at dose levels of 0.5 mg/kg bw/day to dose groups A, C, E, G and H or 20 mg/kg bw/day to groups B, D and F. Groups G and H received the test substance during 14 days. The other groups received a single dose. An overview of the experiments is presented in table 2.1.1-1.

Table 2.1.1-1 overview of dosing and sampling regime

Test group	Number and sex	Dose (mg/kg bw/day)	Comments	Sampling regime
A	6m/6f	0.5 (single oral administration of (3H)-MAB1a)	Pharmacokinetics	Blood samples (pre-dose and 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168h after dosing)
B	6m/6f	20 (single oral administration of (3H)-MAB1a)	Pharmacokinetics	Blood samples (pre-dose and 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 168h after dosing)
C	12m/12f	0.5 (single oral administration of (3H)-MAB1a)	Tissue distribution/excretion	3m/3f at each time point, range of tissues and organs after 3, 6, 24, 168h. Collection of urine and faeces
D	12m/12f	20 (single oral administration of (3H)-MAB1a)	Tissue distribution/excretion	3m/3f at each time point, range of tissues and organs after 3, 12, 60, 168h. Collection of urine and faeces
E	4m/4f	0.5 (single oral administration of (3H)-MAB1a)	Biliary excretion	Bile was collected pre-dose and 1, 2, 3, 4, 6, 10, 12, 24, 48h after dosing; urine and faeces were collected pre-dose and 6, 12, 24, 48h after dosing
F	4m/4f	20 (single)	Biliary excretion	Bile was collected pre-dose and 1, 2, 3, 4, 6, 10, 12, 24, 48h after dosing; urine and faeces were collected pre-dose and 6, 12, 24, 48h after dosing
G	6m/6f	0.5 (repeated oral administration of (3H)-MAB1a for	Pharmacokinetics	Blood samples (pre-dose and 23, 95, 167, 239, 312, 313, 314, 316, 318, 324, 336, 360, 384, 408, 432, 456, 480h after the first dose)

		14 days)		
H	12m/12f	0.5 (repeated oral administration of (3H)-MAB1a for 14 days)	Tissue distribution/excretion	3m/3f at each time point, range of tissues and organs after 1, 6, 42, 168h. Collection of urine and faeces

All samples were counted for radioactivity by liquid scintillation counting (LSC).

Metabolite characterisation was performed in samples (pooled by group and sex and subjected to extraction) of urine, faeces, bile, plasma and tissues. Metabolites were isolated by HPLC and identified by co-chromatography with authentic reference standards.

Results

Concentration profiles of MAB1a in whole blood and plasma were similar. Results of the pharmacokinetic studies are presented in table 2.1.1-2.

Table 2.1.1-2. Pharmacokinetic data (plasma) after single low and high oral dose administration and following repeated oral low dose administration of [^{3}H]-MAB1.

Group and dose	**Group A (single dose, 0.5 mg/kg bw)**		**Group B (single dose, 20 mg/kg bw)**		**Group G (repeated dose, 0.5 mg/kg bw)**	
sex	**m**	**f**	**m**	**f**	**m**	**f**
C_{max} (µg equiv/mL)	0.03	0.02	1.20	0.64	0.04	0.03
T_{max} (h)	6.7	5.3	15.0	10.7	317.3	318.5
$T_{1/2}$ (h)	27.3	19.5	36.3	35.3	34.8	23.9
$AUC_{0-\infty}$ (µg equiv.h/mL)	0.87	0.61	69.8	44.6	0.83	0.57

Following low dose administration (0.5 mg/kg bw, both single and repeated dose), the half live of MAB1a was greater in males than in females. Administration of a single high dose (20 mg/kg bw) resulted in a dose-proportional increase (40-fold) in C_{max}, with maximum concentration reached after 15 (m) and 11 (f) hours. The half live of MAB1a was independent of sex in the high dose groups. AUC-values were supra-proportional to dose, being 80 (m) to 73 (f) fold greater than for 0.5 mg/kg bw.

Repeated dose administration of MAB1a showed steady-state conditions after the 7th-8th dose (as shown by plasma half lives, plasma concentrations and AUC values over the dosing interval).

After single low or high dose administered MAB1a, the majority of MAB1a was excreted by faeces (about 89%, over 168h). Renal elimination accounted for less than 0.3% (low dose) and 0.2% (high dose) of the administered dose. At the low dose, more than 80% of the dose was excreted within two days by both sexes. In the high dose group it took longer to excrete this percentage of administered MAB1a. As with both single dose groups, also after repeated administration of MAB1a radioactivity was mainly excreted by the faeces (80-99%% at t=480h).

Less than 3% of the administered dose (low and high dose) was excreted in bile. Biliary excretion was not complete at t=48h (end of the study). More than 20% of the dose was retained in the carcass (minus GIT and contents) and liver, suggesting that at least 20% of the dose was absorbed.

After oral administration of [^{3}H]-MAB1a, radiolabel was observed in all organs/tissues sampled, irrespective of dose level or regime. In the high dose and the repeated dose groups, residues in all tissues were higher than after a single low dose. After an initial increase of radioactivity in organs/tissues, decreased levels of radioactivity were observed at t=168h after both single (low and high) and repeated low oral dose.

After single oral dose of 0.5 mg/kg bw, low levels of radioactivity were observed in tissues/organs at t-168h (< 0.04 µg equiv/g tissue), except in pituitary ((m) 0.35 µg equiv/g tissue) and Harderian gland ((m) 0.74 and (f) 0.66 µg equiv/g tissue).

After repeated oral dosing of 0.5 mg/kg bw/day highest levels of radioactivity at t=168h were observed in Harderian gland (7.7 (m) and 4.4 (f) µg equiv/g tissue) and pituitary (8.3 (m) µg equiv/g tissue), and 0.1-0.4 µg equiv/g tissue in adrenals, thyroid, spleen, lung, bone marrow, sublingual glands, thymus, liver, brown fat and female pituitary.

After a single oral dose of 20 mg/kg bw, highest amounts of radioactivity at t=168h were observed in Harderian gland (100 (m) and 64 (f) µg equiv/g tissue), pituitary (9.4 (m) and 9.1 (f) µg equiv/g tissue) and adrenals (4.8 (m) and 4.4 (f) µg equiv/g tissue). Relevant amounts (1 to 3 µg equiv/g tissue) were also found in thyroid, spleen, lung, bone marrow, sublingual glands, thymus, brown fat, kidney, liver, ovary and testes.

After oral administration of MAB1a, one metabolite, AB1a, was identified. In plasma, 78-100% of the radioactivity was MAB1a and 5-18% AB1a, for both sexes. In urine, the major portion of radioactivity was associated with very polar material, and only small amounts of radioactivity (0.05%) were associated with MAB1a and AB1a. In urine of both sexes, more parent compound than metabolite was observed, but compared to males, females excreted more metabolite.

In faeces mainly parent compound MAB1a was observed (66-95%), while metabolite AB1a was present for 2-22% (both sexes).

In bile, 68% (m) and 49% (f) was excreted as MAB1a at the lower dose, whereas AB1a accounted for 9% and 14% in males and females, respectively. A high proportion of the radioactivity in bile was associated with very polar material. In bile of the high dose group, the polar material represented a higher proportion of the radioactivity in the sample when compared to the lower dose group.

In organs/tissues more parent compound MAB1a was observed than metabolite AB1a, both after single oral administration of 20 mg/kg bw and repeated oral adminstration of 0.5 mg/kg bw/day. Generally, of the % radioactivity in the organs/tissues 60-90% was present as parent compound and 5-26% as metabolite.

Acceptability

MAB1a content of MK-0244 was 89% (should be at least 90%, according to the notifier). The study is considered acceptable for the overall toxicological evaluation.

Conclusion

[^{3}H]-MAB1a was absorbed after oral administration to the rat, and distributed into body tissues/organs mainly as parent compound (MAB1a), with females generally having lower levels of radiolabelled material than males after single low and high dose exposure as well as after repeated low dose exposure. Highest tissue residue concentrations were observed in glandular tissue (Harderian gland, pituitary, adrenals, (para)thyroid, sublingual gland), spleen, lungs and liver. Radioactivity profiles showed higher plasma/whole blood levels in males than females. After single low dose administration, Cmax was 0.02-0.03 µg equiv/mL after approximately 6h; plasma half lives were 27.3 h and 19.5 h and AUC's were 0.87 and 0.61 µg equivs.h/mL in males and females, respectively. After single high dose administration, Cmax showed a dose-proportional increase (0.6-1.2 µg equiv/mL) and was reached after approximately 13h, but AUC values were supra-proportional to dose (69.9 and 44.6 µg equivs.h/mL in males and females, respectively). MAB1a radioactivity was mainly excreted by the faeces (t=168h, ~ 90%). Less than 3% of the administered dose (low and high) was excreted in bile. After oral administration, one metabolite, AB1a, was identified, which was present for 5-18% in plasma, 2-22% in faeces and 5-26% in organs.

2.1.2 STUDY 2 - Absorption, distribution, metabolism and excretion

Characteristics

Type of study	:	Absorption, distribution, metabolism and excretion	Exposure	:	Single or repeated by gavage; single intravenous
Year of execution	:	1990-1992	Doses	:	I. single oral high dose (20 mg/kg bw) II. multiple oral low dose (0.5 mg/kg bw/day) III. single oral or i.v. low dose (0.5 mg/kg bw)
Test substance	:	MK-0244 (L-656,748-038W002, 91.1% MAB1a and 5.1% MAB1b) [3,7,11,13,23-^{14}C]-MAB1a benzoate (radiochemical purity around 94%; spec. act. 28.82 µCi/mg) [5-^{3}H]-MAB1a (radiochemical purity around 97%; spec. act. 12.27 mCi/mg (benzoate)	Vehicle	:	Propylene glycol/saline (1:1 v/v)
Route	:	Oral and intravenous	GLP statement	:	yes
Species	:	Rat, Sprague Dawley	Guideline	:	Comparable to OECD 417
Group size	:	I. 6/sex II. 6/sex III. 12/sex/route of administration	Acceptability	:	acceptable

Study design

An overview of the experiments is presented in table 2.1.2.

Table 2.1.2 overview of dosing and sampling regime

Test group	Number and sex	Route and dose level (mg/kg bw/day)	Sampling regime
I	6m/6f	Single oral dose of [^{3}H or^{14}C]-MAB1a benzoate at 18.7/20.5 mg/kg bw (m) and 18.8/20.6 mg/kg bw (f)	Urine and faeces collection (at 8 hours, 1, 2, 3, 4, 5, 6, 7 days after dosing) Sacrifice and tissue collection on day 7
II	6m/6f	Pretreatment with daily oral doses of unlabelled MAB1a benzoate at 0.5 mg/kg bw/day for 14 days followed by a single oral dose of [^{14}C]-MAB1a benzoate at 0.5 mg/kg bw	Urine and faeces collection (at 8 hours, 1, 2, 3, 4, 5, 6, 7 days after dosing) Sacrifice and tissue collection on day 7
III	12m/12f	Single oral dose of [^{14}C]-MAB1a benzoate at 0.5 mg/kg bw	Blood samples (at 2, 4, 8, 12, 18 hours (6m/6f) and 1, 2, 3, 4, 5, 6, 7 days (6m/6f) after dosing) Urine and faeces collection (at 8 hours, 1, 2, 3, 4, 5, 6, 7 days after dosing) Sacrifice and tissue collection on day 1 (6m/6f) or day 7 (6m/6f)
	12m/12f	Single intravenous dose of [^{14}C]-MAB1a benzoate at 0.5 mg/kg bw	

Control animals (1/sex/dose group) were not dosed, except control animals in group II which were pre-treated with unlabelled MK-0244, but did not receive a radiolabelled dose.

Samples of blood, urine, and faeces were counted for radioactivity by liquid scintillation counting (LSC) either directly or following sample oxidation. From rats terminated after 7 days blood, bone, brain, fat, heart, kidney, liver, lung, muscle, spinal cord, spleen, testis or ovary and uterus, gastrointestinal tract and carcass were analysed for radioactivity. From rats sacrificed after 24h blood, brain, fat, kidney, liver, muscle, spinal cord and carcass were analysed for radioactivity.

Metabolites (in faeces, liver, kidney, muscle and fat samples) were isolated by HPLC and identified by co-chromatography with reference standards, UV spectrometry, mass spectrometry and NMR.

Results

Absorption

Pharmacokinetic parameters following single oral or intravenous dose of [^{14}C]-MAB1a at 0.5 mg/kg bw are given in the table below.

Dose route (group)	**Single oral dose (III)**		**Single i.v. dose (III)**	
Dose (mg/kg bw)	**0.50**		**0.50**	
sex	**m**	**f**	**m**	**f**
AUC (ppb/h)	893	1127	1635	1517
Half life[a], $T_{1/2}$ (h)	~28 and ~68	~14 and ~76	~28 and ~72	~18 and ~80
Bioavailability[b](%)	54.6	74.3	-	-

a: According to the study author, mean half life after oral dosing was calculated to be 34.4. and 51.1 h in males and females, respectively, assuming first order elimination kinetics.

b: bioavailability (%) = 100x(AUC (oral)/ AUC (i.v.))

Plasma concentrations of radioactivity reached a maximum of 17 ppb after 12 h and of 21 ppb after 4 h in males and females, respectively, and declined to background levels after 96 h in both sexes.

Graphics of plasma radioactivity concentration versus time after dosing indicate the involvement of two phases, resulting in a steep elimination curve the first 24 hours after dosing followed by a slower elimination curve thereafter. This observation was most prominent in females. Plasma elimination half live after low oral dosing were ~28 h and ~68 h for males and ~14 h and ~76 h for females. Plasma elimination half lives after low i.v. dosing were ~28 h and ~72 h for males and ~18 h and ~80 h for females.

Comparison of the AUC values following i.v. and oral dosing showed higher bioavailability of MAB1a benzoate in females (74%) than in males (55%).

Tissue distribution

Following a single high oral dose highest tissue residues after 7 days were observed in lung, followed by spleen, GI-tract, kidney and liver. No differences between sexes and radioactivity label (^{14}C en ^{3}H) were observed. On day 7 after a single low oral dose, highest levels of radioactivity were observed in spleen, followed by lung, GI-tract, ovary, kidney, testes and liver, with males having 2-3 times higher levels of radioactivity than females. Compared to single low oral exposure, radioactivity levels in organs/tissues were supra-proportional after single high oral exposure (m: 2-3 times, f: 4-9 times).

After repeated low oral dosing organs of males showed about 4 times higher levels of radioactivity than females (blood, fat and spinal cord showed only slightly higher levels). Compared to single low oral dose, 1.5-3 times higher levels were observed in organs/tissues after repeated oral dose. Highest levels of radioactivity were observed in spleen, followed by lung, GI-tract, fat, kidney and liver.

Comparing radiolabel distribution in organs/tissue 1 and 7 days after low dose oral administration shows a more pronounced sex-difference and (generally about 100 times) lower levels of radioactivity after 7 days than after 1 day. After low dose i.v. administration (mainly about 60 times) lower levels of radioactivity were found after 7 days than after 1 day.

Comparing data of low dose oral and i.v. administration, shows that the mean tissue radioactivity level in orally exposed males is in accordance with an oral bioavailibility of about 55%. In females, however, at day 7 lower tissue radioactivity levels (about 2-times) were observed in orally exposed females than calculated from i.v. data with 74% oral bioavailability, indicating lower than 74% bioavailability for females.

Excretion

Seven days after treatment with radiolabelled MAB1a benzoate (independent of dose, the route of administration or pre-treatment) excretion was virtually complete, with almost all radioactivity excreted in faeces (>94% within 96h), and only minor amounts (0.1-0.3%) excreted in urine or observed in tissues (0.07-1.6%). Compared to females, males excreted slightly more radioactivity in urine, and showed more radioactivity in tissues.

The majority of the administered radioactivity excreted in faeces was parent MAB1a. In faeces and tissues, one metabolite, AB1a was identified (metabolites were not profiled in urine, due to low levels of radioactivity in urine), and accounted for 0.04-2.2% of the doses on days 1, 3 and 7 after exposure. After single low dose treatment (both oral and i.v.) faeces of males showed higher levels of AB1a than females.

Also small amounts of polar metabolites were detected in faeces and tissues.

As in faeces, also the majority of tissue radioactivity was parent MAB1a, and the only significant metabolite was identified as AB1a. The % radioactivity identified as AB1a in liver, kidney, muscle and fat was 4-23% (all treatments). Compared to i.v. treatment, the % metabolite (day 1) in liver and kidney was 1.5-2 times higher after oral exposure. High dose treatment showed comparable % of metabolite (day 7) in liver, kidney and muscle (~19%), whereas in fat the % metabolite was 2-3 times higher than in females.

Acceptability

The study is considered acceptable for the overall toxicological evaluation.

Conclusion

After single oral administration of 0.5 mg/kg bw, bioavailability was about 55% in male rats and slightly higher in female rats. Plasma elimination half lives in males were about 28h and 68h for the rapid and slow elimination phase, respectively. In females, plasma elimination half lives were about 14h and 76h for these phases, respectively. Comparable elimination half lives were observed after single i.v. administration of 0.5 mg/kg bw. Organs with highest level of radioactivity were spleen, lungs, GI-tract, kidney and liver, with higher tissue residue levels in males compared to females. Compared to single low oral exposure, radioactivity levels in organs/tissue were supra-proportional after single high oral exposure. The majority of administered radioactivity was excreted as parent compound in faeces. In faeces and tissues, one metabolite was identified, AB1a, accounting for up to 2.2% (faeces) and 4-23% (organs) of the administered dose.

2.1.3 STUDY 3 - disposition

Characteristics

Type of study	:	disposition	Exposure	:	Single
Year of execution	:	2004-2005	Doses	:	0.5 mg/kg bw
Test substance	:	[23-^{14}C]-emamectin B1a (radiochemical purity >97%; spec. act. 2.13 MBq/mg)	Vehicle	:	Propylene glycol/physiological saline (50:50 v/v)
Route	:	intravenous	GLP statement	:	yes
Species	:	Rat, Crl:CD(SD)BR	Guideline	:	OECD 417
Group size	:	10/sex	Acceptability	:	acceptabe

Study design

Table 2.1.3 overview of dosing and sampling regime

Test group	Number and sex	Termination	Sampling regime
Bile duct cannulation	3m/4f	t=72h (1m at t=18h due to	Urine and bile collected at 6, 24, 48 and 72 hours after dosing (or at termination time)

		poor bile flow; 1f at t=48h and 2f at 52h due to leakage of infused bile into the body cavity)	Faeces collected at 24, 48 and 72 hours after dosing (or at termination time) At termination collection of blood sample, the gastrointestinal tract plus content and residual carcass
autoradiography	6m/6f	t=2h (1m/1f) t=24h (1m/1f) t=72h (1m/1f) (no data reported for the other rats due to sample loss)	At termination, collection of blood sample, the gastrointestnal tract, including contents, and the residual carcass

Following dosing of the bile duct cannulated rats, samples were analysed for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation.

Following dosing of the non-cannulated rats, the distribution of radioactivity in the contents and wall of stomach, duodenum, jejenum, ileum, caecum, colon and rectum were determined.

Results

Small amounts of blood were observed in the urine of some animals after dosing.

Following intravenous administration of [^{14}C]-emamectin B1a at 0.5 mg/kg bw to bile duct cannulated rats, the total % recovery of administered radioactivity after 72h was 95-97% and 96% in male rats and the female rat, respectively. Faecal excretion was the major route of elimination, with 36-38% and 44% of the dose after 72h in male rats and the female rat, respectively. Biliary elimination accounted for 11-17% in male rats and 6% in the female rat. Urinary excretion accounted for 1.4-1.5% in male rats and 0.6% in the female rat. The overall rate of excretion of radioactivity was not different for the two male rats and one female rat. However, elimination by bile and urine was more extensive in male rats. After 72h, excretion was not complete and 34-37% and 37% of the dose was observed in the carcasses of male rats and the female rat, respectively.

Autoradiography of the gastro-intestinal tract of non-cannulated rats showed that 2h after intravenous administration of [^{14}C]-emamectin B1a the highest amount of radioactivity was in the contents of the small intestine, and radioactivity levels were lower in the contents of the large intestine. Highest level of radioactivity was observed in the contents of the ileum. At 24h after dosing, the radioactivity had increased in the content of the large intestine and decreased in the content of the small intestine. At 72h after dosing most of the administered radioactivity had been excreted, leaving low levels of radioactivity in the gastrointestinal tract and contents, with the highest level of radioactivity in the content of the large intestine.

Acceptability

Although data were only from a limited number of animals, the study is considered acceptable for the overall toxicological evaluation.

Conclusion

Administration of a single intravenous dose of 0.5 mg/kg bw [^{14}C]-emamectin B1a to rats showed similar overall excretion rates for male and female rats. However, a sex difference was observed in the route of excretion with males having more urinary and biliary excretion than the female rat. The major route of elimination was excretion in faeces, acounting for 36-38% and 44% of the dose after 72h in male rats and the female rat, respectively. Biliary excretion accounted for 11-17% in male rats and 6% in the female rat and urinary excretion accounted for 1.4-1.5% in male rats and 0.6% in the female rat. In the carcass, 34-37% and 37% of the dose was observed in male rats and the female rat, respectively. Autoradiography after intravenous administration showed that emamectin is taken up by the gastro-intestinal tract and mainly eliminated via intestinal secretion and subsequent excretion in faeces.

3 HEALTH HAZARDS

Emamectin was originally developed as the hydrochloride salt MK 243 (L-656,748-010V), but was subsequently changed to MK 244, the benzoate salt (L-656,748-038W), and benzoate hydrate (L-656,748-052S) because of superior storage and handling characteristics. There is no complete toxicity data set available for each salt. For the present evaluation, the available toxicity data are a composition of studies performed with abovementioned three emamectin salts, since the notifier considers these three emamectin salts as toxicologically bioequivalent. Moreover, in the EFSA conclusion (EFSA Journal 2012;10(11):2955) the following is stated: *Toxicological studies were performed with different emamectin salts: hydrochloride salt, the benzoate salt and the benzoate hydrate salt. The three salts are considered toxicologically equivalent.*

The studies reported, with the exception of those employing molar concentrations, were performed using dose levels calculated as 'base compound' to account for differences in the molecular weights of the salts. The factors applied are indicated in the studies, and were generally 1.04 (hydrochloride), 1.14 (benzoate) and 1.16 (benzoate hydrate).

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 STUDY 1 - Acute toxicity, up & down procedure

Study reference: B.6.2.1.1, STUDY 1

Characteristics

Type of study	:	Acute toxicity, up & down procedure	Exposure	:	Single dose, gavage
Year of execution	:	2006	Dose	:	20.8, 66, 208, and 658 mg/kg bw*
Test substance	:	Emamectin technical, benzoate salt Batch: SNA6B019 Purity: 96.2% (MK244)	Vehicle	:	0.5% w/w CMC solution in water
Route	:	oral	GLP statement	:	Yes
Species	:	Rat; Sprague Dawley albino	Guideline	:	OECD 425
Group size	:	1-3 females /dose	Acceptability	:	Acceptable
			LD50 rats	:	208 mg/kg bw * conf. interval: 60.9-622 mg/kg bw

* Dose levels are expressed as base compound (factor 1.14).

Study design

The study is in accordance with OECD 425.

Total of animals tested: one rat at 20.8 mg/kg, two rats at 66 mg/kg, 3 rats at 208 mg/kg and 1 rat at 658 mg/kg.

The test substance was administered as a 6% w/v suspension in a 0.5% w/w solution of CMC in destilled water.

Results

Mortality: In the up and down procedure the first rat tested at 208 mg/kg died, the 2nd survived and the 3rd died. Also the rat dosed with 658 mg/kg died.

Symptoms of toxicity: Signs of toxicity were found in the 208 and 658 mg/kg dose groups: hypoactivity, tremors, soft faeces and/or reduced faecal volume, diarrhoea, ano-genital staining.

Body weight: During the first week the surviving animal at 208 mg/kg dose level lost weight, but gained weight over the remaining week of the study. Animals of the lower dose groups gained weight over the total period of the study.

Pathology: Discolouration of intestines was found in the decedents of the 208 and 658 mg/kg dose groups.

Acceptability

The study is considered acceptable.

Conclusions

The estimated acute oral LD50 for emamectin benzoate salt is 237 mg/kg bw for females. When expressed as base compound (dividing by a factor of 1.14) the LD50 is 208 mg/kg bw with a confidence interval of 60.9 – 622 mg/kg bw.

3.1.1.2 STUDY 2 - Acute toxicity

Study reference: B.6.2.1.1, STUDY 2

Characteristics

Type of study	:	Acute toxicity	Exposure	:	Single dose, gavage
Year of execution	:	1988	Dose	:	44.4, 66.6, 100, 150, and 225 mg/kg bw **
Test substance	:	L656,748-010V003 Hydrochloride salt Purity: 96.9% *	Vehicle	:	Water
Route	:	Oral	GLP statement	:	Yes***
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	In accordance with OECD 401
Group size	:	5/sex	Acceptability	:	Acceptable
			LD50 rats	:	88 mg/kg bw (m) 76 mg/kg bw (f)

* 92.8% L-656,748 B1a and 4.1% B1b; 0.76% (w/w) propylgallate added as an antioxidant.
** dose levels are expressed as base compound (factor 1.04).
*** in the Lankas (1994a) study: GLP statement is of 1994 whereas the study is performed in 1988! (QA statement is from 1988); in the Lankas (1992f) study GLP statement is of 1993 and QA statement of 1988.

Study design

The study is in accordance with OECD 401.

Results

Mortality: Death occurred from day 1-5 in the high dose group and from day 4 -12 in all other groups. Rats were moribund with loss of righting reflex before death.

Dose group mg/kg bw	44.4	66.6	100	150	225
Males	0	1	3	5	5

Females	0	2	4	5	5

Symptoms of toxicity: Clinical signs were similar for both sexes. At all dose levels mucous-like stools were noted 2-4 hours after dosing. Ataxia and whole body tremors occurred in all animals of all dose groups within 4 hours after exposure in the high dose groups (150 and 225 mg/kg) and starting after 2 days in the low dose groups and persisted sometimes several days. Bradypnoea was observed in some animals in the low dose groups and nearly all animals of the high dose groups.

Irritability, salivation and lacrimation were seen at most dose levels from the 2nd to the 3rd or 4th day. Also decreased activity was observed in several animals.

Body weight: Unaffected in low dose group. At higher dose levels (66.6 mg/kg and above) animals showed weight loss or slower weight gain during first week. Weight gain resumed in second week.

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD50 for emamectin (HCl salt) is 88 mg/kg bw for males and 76 mg/kg bw for females.

3.1.1.3 STUDY 3 - Acute toxicity

Study reference: B.6.2.1.1, STUDY 3

Characteristics

Type of study	:	Acute toxicity	Exposure	:	Single dose, gavage
Year of execution	:	1995	Dose	:	32, 41.6, 54.1, 70.3, and 91.4 mg/kg bw*
Test substance	:	MK 0244 L656,748-052S lot #5 Benzoate hydrate salt Purity: 97.8%	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	In accordance with OECD 401
Group size	:	5 /sex/dose	Acceptability	:	Acceptable
			LD50 rats	:	63 mg/kg bw (m) 76 mg/kg bw (f) 70 mg/kg bw (combined)

* dose levels are expressed as base compound (factor 1.16).

Study design

The study is in accordance with OECD 401.

Results

Mortality: Death occurred from day 3-6.

Dose group (mg/kg bw)	**32**	**41.6**	**54.1**	**70.3**	**91.4**
Males	0	0	2	3	4
Females	0	0	0	1	5

Symptoms of toxicity: Clinical signs were similar for both sexes. Tremors occurred in all animals of all dose groups. Bradypnoea, ptosis and decreased activity were observed in the 41.6 mg/kg dose group and higher, and lateral recumbency, urine staining and red discharge of nose and eyes were observed at the two highest dose levels.

Body weight: Compared to the low dose group, a dose dependent decrease in body weight gain was observed in the first week after dosing. Body weight losses were not observed.

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD50 for emamectin (benzoate hydrate salt) is 63 mg/kg bw for males and 76 mg/kg bw for females.

The combined LD50 was 70 mg/kg bw.

3.1.1.4 STUDY 4 - Acute toxicity bioequivalence study

Study reference: B.6.2.1.1, STUDY 4

Characteristics

Type of study	:	Acute toxicity bioequivalence study	Exposure	:	Single dose, gavage
Year of execution	:	1992-1993	Dose	:	40, 60, 90 and 135 mg/kg bw*
Test substance	:	MK 0244 L656,748-038W lot #2 (benzoate-methyl t-butyletherate solvate; purity 96.4 %) and L656,748-052S lot #2 (Benzoate hydrate salt; purity 99.1%)	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	no guideline, but resembles OECD 401
Group size	:	5 females/group	Acceptability	:	Acceptable
			LD50 rats		
			038W	:	53 mg/kg bw (f)
			052S	:	58 mg/kg bw (f)

* all dose levels are expressed as base compound: factor 1.14 for 038W and factor 1.16 for 052S.

Study design

The study is in accordance with OECD 401, except that 2 different active substances were tested in the same study.

Results

Mortality: Death occurred from day 2-6.

Dose group (mg/kg bw)	**40**	**60**	**90**	**135**

038W	0	4	5	5
052S	0	3	5	5

The incidence of mortality for both solvates showed no significant difference (calculated by the study authors conform the Mantel Haenszel procedure: J. Natl. Cancer Inst. 22: 719-748, 1959).

Symptoms of toxicity: Clinical signs were similar for both solvates. Most effects were seen at dose levels of 60 mg/kg and higher: bradypnoea, decreased activity, ptosis, salivation, laying on one side, blood-like staining of eyes, nose or mouth, urine staining, and loss of righting reflex. Tremors and ataxia were observed already in the 40 mg/kg dose group. Some of the effects like bradypnoea, loss of righting reflex and ptosis started for 038W at a lower dose level than for 052S, whereas decreased activity was seen at a lower dose level for 052S.

Body weight: A slight decrease in body weight gain was observed in the 60 mg/kg bw dose groups in the first week after dosing compared to the low dose group. Body weight losses were not observed (only based on 1 surviving animal for 038W, and 2 surviving animals for 052S).

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The acute toxicity in female rats of L-656,748-038W (emamectin benzoate) and L-656,748-052S (emamectin benzoate hydrate) is similar.

3.1.1.5 STUDY 5 - Acute toxicity study

Study reference: B.6.2.1.1, STUDY 7

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	Single dose, gavage
Year of execution	:	1992	Dose	:	40, 68, 116, 196, and 334 mg/kg bw*
Test substance	:	MK 0244 L656,748-038W lot #2 (benzoate-methyl t-butyletherate solvate; purity unknown) and L656,748-052S lot #1 (Benzoate hydrate salt; purity unknown)	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	no
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	no guideline, but resembles OECD 401
Group size	:	5 females/group	Acceptability	:	Acceptable
			LD50 rats		
			038W	:	89 mg/kg bw (f)
			052S	:	89 mg/kg bw (f)

* all dose levels are expressed as base compound: factor 1.14 for 038W and factor 1.16 for 052S.

Study design

The study resembles OECD 401, except that 2 different active substances were tested in the same study. Only for mortality the individual results were presented, and no pathology was performed. There was no information on housing conditions.

Results

Mortality: Death occurred within 24 h after dosing up to day 6.

Dose group (mg/kg bw)	40	68	116	196	334
038W	0	1	4	5	5
052S	0	0	5	5	5

Symptoms of toxicity: Clinical signs were reported to be similar for both salts and occurred at all dose levels within 2-5 h after dosing. Effects observed were: tremors, ataxia, and decreased activity. Further, irritability, bradypnoea and salivation were observed from 68 mg/kg bw (irritability already seen at 40 mg/kg bw for compound 038) and lacrimation and loss of righting reflex were observed at 116 mg/kg bw and higher. Surviving animals appeared normal by day 8 or 9.

Body weight: It was reported that no apparent effects on body weight were seen.

Pathology: Not performed.

Acceptability

The study is considered acceptable.

Conclusions

The acute toxicity in female rats of L-656,748-038W (emamectin benzoate) and L-656,748-052S emamectin benzoate hydrate) is similar.

3.1.1.6 STUDY 6 - Acute toxicity

Study reference: B.6.2.1.1, STUDY 6

Characteristics

Type of study	:	Acute toxicity	Exposure	:	Single dose, gavage
Year of execution	:	1988-92	Dose	:	20, 30, 45, 67.5 and 101.2 mg/kg bw **
Test substance	:	L656,748-010V003 Hydrochloride salt Purity: 96.9% *	Vehicle	:	Water
Route	:	Oral	GLP statement	:	Yes***
Species	:	mouse Crl:CF-1 BR strain	Guideline	:	In accordance with OECD 401
Group size	:	5 /sex/dose	Acceptability	:	Acceptable
			LD50 mice	:	22 mg/kg bw (m) 31 mg/kg bw (f)

* 92.8% L-656,748 B1a and 4.1% B1b; 0.76% (w/w) propylgallate added as an antioxidant.
** dose levels are expressed as base compound (factor 1.04).
*** GLP statement- is of 1993 and QA statement of 1988.

Study design

The study is in accordance with OECD 401.

Results

Mortality: Death occurred from 33 minutes to 7 days and was preceded by bradypnoea and loss of righting reflex.

Dose group mg/kg bw	20	30	45	67.5	101.2
Males	2	4	5	5	5
Females	3	0	4	4	5

Symptoms of toxicity: Clinical signs were similar for both sexes. Ataxia and whole body tremors occurred in all animals of all dose groups within 2 hours after exposure and persisted several days.

Body weight: Generally unaffected.

Pathology: No data.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD50 for emamectin (HCl salt) is 22 mg/kg bw for males and 31 mg/kg bw for females.

3.1.1.7 STUDY 7 - Acute toxicity study

Study reference: B.6.2.1.1, STUDY 7

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	Single dose, gavage
Year of execution	:	1992	Dose	:	5, 10, 20, 40, and 80 mg/kg bw* (1st study) 80, 144, 259, and 466 mg/kg bw * (2nd study)
Test substance	:	MK 0244 L656,748-038W lot #2 (benzoate-methyl t-butyletherate solvate; purity unknown) and L656,748-052S lot #1 (Benzoate hydrate salt; purity unknown)	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	no
Species	:	Mice Crl:CD-1(ICR) BR strain	Guideline	:	-
Group size	:	5 females/group	Acceptability	:	Acceptable
			LD50 mice		
			038W	:	120 mg/kg bw (f)
			052S	:	107 mg/kg bw (f)

* all dose levels are expressed as base compound: factor 1.14 for 038W and factor 1.16 for 052S.

Study design

The study resembles OECD 401, except that 2 different active substances were tested in the same study. Only for mortality the individual results were presented, and no pathology was performed. There was no information on housing conditions.

Results

Mortality: No mortality occurred in the first study up to and including 80 mg/kg bw. The mortality in the second study is presented in the table. Death occurred within 24 h after dosing up to day 7 in mice treated with 052S and up to day 10 when treated with 038W.

Dose group (mg/kg bw)	80	144	259	466
038W	0	4	5	5
052S	1	4	5	5

Symptoms of toxicity: No clinical signs were observed in mice dosed up to 40 mg/kg bw. Clinical signs were similar for both salts. Tremors, ataxia, bradypnoea and decreased activity were seen from day 2 of dosing at 80 and 144 mg/kg bw, and already after 2 hours at higher dose levels. Other effects at these higher dose levels were ptosis and loss of righting. Surviving animals appeared normal by day 7.

Body weight: Marked body weight losses were reported for mice treated with 038W at 259 and 466 mg/kg bw and still alive at day 7. Surviving mice given 144 mg/kg bw showed slight body weight losses during the first week, but recovered most of the weight loss by day 14.

Pathology: Not performed.

Acceptability

The study is considered acceptable.

Conclusions

The acute toxicity in female mice of L-656,748-038W (emamectin benzoate) and L-656,748-052S (emamectin benzoate hydrate) is similar.

3.1.1.8 STUDY 8 - Acute toxicity study

Study reference: B.6.2.1.1, STUDY 8

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	Single dose, gavage
Year of execution	:	1994	Dose	:	70, 120, 192, and 307 mg/kg bw *
Test substance	:	MK 0244 L656,748-052S lot #2 Benzoate hydrate salt; Purity: 97.6%	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	yes
Species	:	Mice Crl:CD-1(ICR) BR strain	Guideline	:	-
Group size	:	5/sex/dose, except high dose group: 5 f only	Acceptability	:	Acceptable
			LD50 mice	:	134 mg/kg bw (m) 156 mg/kg bw (f) 145 mg/kg bw (combined)

* dose levels are expressed as base compound (factor 1.16).

Study design

The study is in accordance with OECD 401, except that food was withheld only 2 h before dosing instead of overnight.

Results

Mortality: Death occurred in the high dose group on day 2 (in a single animal on day 5). At 192 mg/kg bw mortality occurred between days 2 and 10.

Dose group (mg/kg bw)	70	120	192	307
Males	1	0	5	-
Females	0	0	4	5

Symptoms of toxicity: Tremors were observed in all animals during the first week and in surviving animals of the 120 mg/kg dose group and higher during the second week. Other signs of toxicity were ataxia, bradypnoea, ptosis, decreased activity, and lateral recumbancy. Unkempt coat was observed in some animals of the 120 and 192 mg/kg dose group.

Body weight: Marked body weight losses during the first week were found for the 192 mg/kg dose group. In the 120 mg/kg bw dose group, body weight losses of about 11% were observed during the first week, but mice regained most of the weight loss by day 14. Slight decreases in bodyweight were observed in several animals of the low dose group.

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The acute oral LD50 for emamectin (benzoate hydrate salt) is 134 mg/kg bw for males and 156 mg/kg bw for females.

The combined LD50 was 145 mg/kg bw.

3.1.1.9 STUDY 9 - Acute toxicity study

Study reference: B.6.2.1.1, STUDY 9

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	Single dose, gavage
Year of execution	:	1992	Dose	:	60, 90, 135, and 202 mg/kg bw*
Test substance	:	MK 0244 L656,748-038W lot #2 (benzoate-methyl t-butyletherate solvate; purity 96.4%) or L656,748-052S lot #2 (Benzoate hydrate salt; purity 99.1%)	Vehicle	:	aqueous methylcellulose
Route	:	Oral	GLP statement	:	yes
Species	:	Mice Crl:CD-1(ICR) BR strain	Guideline	:	-
Group size	:	5 females/group	Acceptability	:	Acceptable
			LD50 mice		
			038W	:	165 mg/kg bw (f)
			052S	:	141 mg/kg bw (f)

* all dose levels are expressed as base compound: factor 1.14 for 038W and factor 1.16 for 052S.

Study design

The study resembles OECD 401, except that 2 different active substances were tested in the same study and that food was withheld only 2 h before dosing instead of overnight.

Results

Mortality: Deaths occurred between day 2 and 12 for compound 038W, and between day 1 and 4 for compound 052S.

Dose group (mg/kg bw)	60	90	135	202
038W	0	0	0	5
052S	0	0	2	5

The incidence of mortality for both solvates showed no significant difference (calculated by the study authors conform the Mantel Haenszel procedure: J. Natl. Cancer Inst. 22: 719-748, 1959).

Symptoms of toxicity: Clinical signs were observed from the 90 mg/kg dose group onwards in both exposed groups and were observed only during the first week, or up to mortality. Clinical signs in these groups were tremors, decreased activity, ataxia, and ptosis. Additional signs of toxicity observed in the two high dose groups were bradypnoea and loss of righting reflex.

Body weight: Marked body weight losses over the first week were found for the two animals of the 038W-high dose group that died in week 2. In the 135 mg/kg bw dose groups, slight weight loss was found in the 052S group and moderate weight loss (17%) in the 038W group. Very slight weight losses were found in most animals of the 90 mg/kg dose groups. All surviving animals regained (most of) the weight loss during week 2.

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The acute toxicity in female mice of L-656,748-038W (emamectin benzoate) and L-656,748-052S (emamectin benzoate hydrate) is similar.

3.1.2 Human data

No data available.

3.1.3 Other data

3.1.3.1 STUDY 1 - Acute neurotoxicity

Study reference: B.6.7.1, STUDY 1

Characteristics

Type of study	:	Acute neurotoxicity	Exposure	:	Single dose by gavage
Year of execution	:	1989-1992	Doses	:	0, 27.4, 54.8, and 82.2 mg/kg bw*
Test substance	:	MK-0243: L-656,748-010V003 hydrochloride salt Purity: 96.9%	Vehicle	:	water
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) Br strain	Guideline	:	-
Group size	:	10 m/f	Acceptability	:	acceptable
			LD50	:	67 mg/kg bw (m)
				:	70 mg/kg bw (f)
			NOAEL	:	< 27.4 mg/kg bw

* dose levels are expressed as base compound (factor 1.04).

Study design

Following a 19-hour fast, animals were given a single oral dose, by gavage, of L-656,748-010V (the hydrochloride salt) dissolved in sterile water at dose levels of 0, 27.4, 54.8 or 82.2 mg/kg at a dose volume of 5 mL/kg.

Morbidity, mortality and clinical observations were recorded frequently on the day of administration, and once or twice daily for 3 weeks thereafter. Body weights were recorded on the day prior to test substance administration and weekly thereafter. On day 21, all survivors were killed and, together with decedents, were subjected to gross examination of the brain (weighed), spinal cord, optic and sciatic nerves. Samples of these tissues from all animals were preserved, processed to haematoxylin and eosin stained sections and examined microscopically. Calculation of the 21-day LD50 values and their 95% confidence limits was made by the method of probit analysis and in the calculation the moribund animals were included.

Results

The results are summarized in table 3.1.3.1-1.

Table 3.1.3.1-1Results from a single dose neurotoxicity test in rats

Dose (mg/kg bw)	**control**		**27.4**		**54.8**		**82.2**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	0	0	0	0	2	2 [a]	8 [a]	7 [a]	dr
Clinical signs: [b]									dr
-tremors			8 +	6 +	10 ++	9 ++	10 ++	10 ++	
-ataxia			3 +	2 +	9 ++	7 +	8 ++	10 ++	
-bradypnoea					3 +	2 +	8 +	7 +	
-salivation				3 +	3 +	6 +	4 +	6 +	
-irritability					10 +	4 +	8 +	6 +	
-decreased activity					6 +	1 +	6 +	2 +	
-reddish discharge around eye, nose and/or mouth			1 +		4 ++	2 ++	8 ++	8 ++	
-loss of righting reflex					4 +	2 +	7 ++	6 ++	
-ptosis					2 +	2 +	1 +		
-hypothermia							1 +	5 +	
-urine stains							2 +	5 +	
Body weight gain (g) during 1st week	+112	+56	+69	+45	+35	+33	+15 [c]	+22	dr
Food consumption	Not performed								
Water consumption	Not performed								
Functional observational battery	Not performed								
Motor/locomotor activity	Not performed								

Dose (mg/kg bw)	control		27.4		54.8		82.2		dr
Sex	m	f	m	f	m	f	m	f	
measurements									
Brain weight	No treatment-related effect								
Neuropathology - macroscopy - microscopy	No gross changes or alterations See text below								

a included are also animals that were killed in moribund condition
b number of animals involved; + clinical sign present; ++ clinical sign present but more severe or during longer period than +
c mean of the only 2 surviving males

Death, or premature sacrifice, was confined to animals administered 54.8 and 82.2 mg/kg bw, and occurred 2 to 6 days after test substance administration. With the exception of salivation occurring 5 - 10 minutes after administration, clinical signs of neurotoxicity were evident from 5 hours - 3 days at 27.4 mg/kg bw and from 5 hours - 9 days at 54.8 and 82.2 mg/kg bw. Reduced weight gain occurred in all treated groups during the week following administration in a dose-related manner. Thereafter, the mean weight gains per week of these groups exceeded that of the controls. However, the overall weight change was still below controls for the mid and high dose females and for males in all treated groups. Very slight to slight degeneration of the white matter in brain and spinal cord and very slight to slight degeneration of the sciatic nerve were observed in both sexes at all dose levels with incidences of 16/20, 18/20 and 15/20, in order of ascending dose. Moderate degeneration of the sciatic nerve was observed in the high dose group only, in 2 male rats. Autolysis of brain and spinal cord, found in 6 dead animals (1m of mid dose and 1f/4m of high dose), precluded histological evaluation. This may have accounted for the lack of a dose-response relationship. Neural lesions were evident 2 days after administration in decedents. Survivors showed lesions in brain, spinal cord, and sciatic nerve, but in the high dose group, lesions predominantly occurred in the spinal cord and sciatic nerve. The alterations were characterised by focal vacuolation of white matter or nerve fibres, accompanied by small numbers of swollen axons or remnants of cell debris. Lesions did not occur in the optic nerve.

Acceptability

Study design resembles OECD 424. Measurement of food consumption, functional observational battery and motor/locomotor activity was not performed. Since this study is only an acute exposure study, the deviations from the OECD 424 protocol are considered acceptable.

Conclusions

The acute oral LD50 (95% confidence limits) for emamectin (HCl salt) for males is 67 (54 – 84) mg/kg bw and for females 70 (55 – 104) mg/kg bw.

A no-effect level was not observed in this study since signs of neurotoxicity as well as histopathological lesions in the brain, spinal cord and sciatic nerve were observed at all dose levels. The lowest effect level was 27.4 mg/kg bw.

3.1.3.2 STUDY 2 - Acute neurotoxicity

Study reference: B.6.7.1, STUDY 2

Characteristics

Type of study	:	Acute neurotoxicity	Exposure	:	Single dose by gavage
Year of execution	:	1989-1992	Doses	:	0, 0.5, 2.5, 5.0, 10, 25 mg/kg bw*
Test substance	:	MK-0243:	Vehicle	:	water

		L-656,748-038W002 benzoate salt Purity: 94.2%			
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) Br strain	Guideline	:	-
Group size	:	10 /sex/dose	Acceptability	:	acceptable
			LD50	:	> 25 mg/kg bw
			NOAEL	:	5 mg/kg bw

* dose levels are expressed as base compound (factor 1.14).

Study design

Following an 18-hour fast, animals were given a single oral dose, by gavage, of L-656,748-038W (the benzoate salt) dissolved in sterile water at dose levels of 0, 0.5, 2.5, 5.0, 10, or 25 mg/kg bw at a dose volume of 5 mL/kg.

Morbidity, mortality and clinical observations were recorded frequently on the day of administration, and once or twice daily for 3 weeks thereafter. Body weights were recorded on the day prior to test substance administration and weekly thereafter. On day 21, all survivors were killed and, together with decedents, were subjected to gross examination of the brain (weighed), spinal cord, sciatic nerves and eyes with optic nerves. Samples of these tissues from all animals were preserved, processed to haematoxylin and eosin stained sections and examined microscopically.

Results

The results are summarized in table 3.1.3.2-1.

Table 3.1.3.2-1 Results from a single dose neurotoxicity test in rats

Dose (mg/kg bw)	**0**		**0.5**		**2.5**		**5**		**10**		**25**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality [a]				1		1							
Clinical signs: (1)													
-Tremors									2 +	2 +	10 ++	10 +	dr
-ataxia				1 +									
-bradypnoea				1 +		1 +							
-salivation													
-irritability									1 +	1 +	7 +	8 +	dr
-decreased activity				1 +		1 +							
-reddish discharge around eye/nose/mouth						1 +							
-urine stains												1 +	
-yellowish brown unformed stools												1 +	
Body weight gain											d	d	
Food consumption	Not performed												
Water consumption	Not performed												
Functional observational battery	Not performed												
Motor/locomotor activity measurements	Not performed												
Brain weight	No treatment-related effects												
Neuropathology													

Dose (mg/kg bw)	**0**		**0.5**		**2.5**		**5**		**10**		**25**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
- macroscopy	No gross changes or alterations												
- microscopy													
Brain: white matter degen.											5	7	
Spinal cord: white matter degen.											10	8	
Sciatic nerve: degen.	2		2	2			2	1	2	1	10	9	

a animals were killed in moribund condition on day 12

(1) number of animals involved; + clinical sign present; ++ clinical sign present but more severe or during longer period than +

Two rats (dose groups 0.5 and 2.5 mg/kg bw) were killed in poor clinical condition with body weight loss. No other deaths occurred.

Clinical signs (tremors and irritability) were confined to animals at 10 and 25 mg/kg bw.

Tremors were evident from 6 hours to 5 days after administration. Since the animals killed prematurely did not exhibit clinical symptoms similar to those at higher dose levels (furthermore, the onset of signs observed in the two moribund animals was from day 8), their poor clinical condition is considered not to be related to test substance administration.

Total weight gain was slightly decreased (11-12%, but not stat. significant) in the high dose group (m/f). Brain weights were unaffected by treatment and no test substance-related gross lesions occurred. A very slight to slight white matter degeneration occurred in the brain and spinal cord at 25 mg/kg bw, which was considered to be related to test substance administration. The effect was characterised by the presence of vacuoles. In the brain these vacuoles sometimes contained macrophages or debris. An increase in the incidence of vacuoles containing debris also occurred in the sciatic nerve at 25 mg/kg bw. Lesions did not occur in the optic nerve at any dose level.

Acceptability

Study design resembles OECD 424. Measurement of food consumption, functional observational battery and motor/locomotor activity was not performed. Since this study is only an acute exposure study, the deviations from the OECD 424 protocol are considered acceptable.

Conclusions

The acute oral LD50 for emamectin (benzoate salt) for males and females is > 25 mg/kg bw.

A no-effect level was established at 5 mg/kg bw, based on clinical signs of neurotoxicity. Histopathological lesions in the brain, spinal cord and sciatic nerve were only observed in animals of the highest dose level of 25 mg/kg bw.

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 STUDY 1 - Acute toxicity

Study reference: B.6.2.1.2, STUDY 1

Characteristics

Type of study	:	Acute toxicity	Exposure	:	Single dose, 24 h
Year of execution	:	2006	Dose	:	877 mg/kg bw (f), and/or 1754 mg/kg bw (m/f)*
Test substance	:	Emamectin technical, benzoate salt Batch: SNA6B019 Purity: 96.2% (MK244)	Vehicle	:	Water
Route	:	dermal	GLP statement	:	Yes
Species	:	Rat; Sprague Dawley albino	Guideline	:	OECD 402
Group size	:	5 /sex/dose	Acceptability	:	Acceptable
			LD50 rats	:	>1754 mg/kg bw (m/f)*

* Dose levels are expressed as base compound (factor 1.14) (when expressed as emamectin salt, the dose levels are 1000 and 2000 mg/kg bw).

Study design

The study is in accordance with OECD 402.

The test substance was a white powder and was applied as a dry paste (75% w/w mixture in destilled water).

Results

Mortality: Two females of the high dose group were euthanized for humane reasons on day 2. They showed mouth discharge and were in moribund condition.

Symptoms of toxicity: Clinical signs were similar for both sexes. All rats at both dose levels showed hypoactivity, tremors and/or ataxia. The females of the 877 mg/kg dose group also showed irregular respiration. All rats recovered by day 11 or 12.

Body weight: During the first week animals lost weight. However, all but 2 females of the high dose group gained weight over the remaining week of the study.

Pathology: In two females of the high dose group the lungs were found extremely red. Further, no gross abnormalities in any of the animals (m/f).

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD50 for emamectin benzoate salt is > 2000 mg/kg bw for males and females. When expressed as base compound (dividing by a factor of 1.14) the LD50 is > 1754 mg/kg bw.

3.2.1.2 STUDY 2 - Acute toxicity study

Study reference: B.6.2.1.2, STUDY 2

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	Single dose for 24h
Year of execution	:	1994-5	Dose	:	2000 mg/kg bw*
Test substance	:	MK 0244 L656,748-052S lot #5	Vehicle	:	-

		(Benzoate hydrate salt; purity 96.4%)			
Route	:	Dermal	GLP statement	:	yes
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	-
Group size	:	5 /sex/dose	Acceptability	:	Acceptable
			LD50 rats	:	> 2000 mg/kg bw

* dose levels are expressed as base compound (factor 1.16).

Study design

The study resembles OECD 402 (limit dose study). Deviations from protocol: The test substance was applied as neat compound on the test site which was moistened with 0.5 mL saline and covered with an occluded dressing. Collars were placed on the animals during the whole study.

Results

Mortality: One male rat died on day 7. This death was considered to be due to oral ingestion since the rat was found without the occlusive dressing on the morning of day 2. According to the study authors it is likely that this rat ingested a lethal dose since the oral lethal dose is about 80 mg/kg bw.

Symptoms of toxicity: Prior to death this animal was observed with tremors, bradypnoea and decreased activity. Tremors were also seen in three of the remaining four males from days 4 or 7, up to day 10, and in one of five females from days 7 to 10. No signs of dermal irritation were seen.

Body weight: A decrease in bodyweight (about 15 %) was observed in only one male and female over the first week. Both animals regained some of the weight loss by day 14.

Pathology: No treatment-related gross findings.

Acceptability

The study is considered acceptable.

Conclusions

The dermal LD50 for emamectin (benzoate hydrate salt) for male and female rats is > 2000 mg/kg bw.

3.2.1.3 STUDY 3 - Acute toxicity

Study reference: B.6.2.1.2, STUDY 3

Characteristics

Type of study	:	Acute toxicity	Exposure	:	Single dose, 24 h
Year of execution	:	2010	Dose	:	439 mg/kg bw, 877 mg/kg bw, and 1754 mg/kg bw*
Test substance	:	Emamectin technical, benzoate salt Batch: SNA6A015 Purity: 96.6% (MK244G)	Vehicle	:	-
Route	:	dermal	GLP statement	:	Yes
Species	:	Rat; CRL:(WI)BR Wistar	Guideline	:	OECD 402
Group size	:	5 /sex/dose	Acceptability	:	Acceptable
			LD50 rats	:	Between 439 and 877 mg/kg bw for males and 1660 mg/kg bw for females*

* Dose levels are expressed as base compound (factor 1.14) (when expressed as emamectin salt, the dose levels are 500, 1000 and 2000 mg/kg bw).

Study design

The study resembles OECD 402. Deviations from protocol: The test substance was a white powder and was applied as neat compound on the test site. However, a solid compound should be moistened sufficiently to ensure good contact with the skin.

Results

Mortality:

Dose group (mg/kg bw)	439	877	1754
Males	1	3	1
Females	0	0	3

Symptoms of toxicity: In all dose groups clinical signs were observed 2 days after the treatment. These included vocalization, irritability, tremors, tonic convulsion, piloerection, decreased activity, hunched back, discharge coloured, nose, area around eyes and incoordination. Additionally, prone position, dyspnoea and laying on the side were noted in some animals dosed at 877 mg/kg and 1754 mg/kg.

Body weight: The majority of surviving animals showed marked bodyweight loss during the first week of the observation period.

Pathology: No gross abnormalities in any of the animals (m/f). No treatment related skin irritation was observed in any animal throughout the study.

Acceptability

The study is considered acceptable.

Conclusions

The acute dermal LD50 for emamectin benzoate salt is between 500 and 1000 mg/kg bw for males and 1893 mg/kg bw for females (although there is no dose-response, the results at 1000 mg/kg bw cannot be dismissed). When expressed as base compound (dividing by a factor of 1.14) the LD50 is between 439 and 877 mg/kg bw for males and 1660 mg/kg bw for females.

3.2.2 Human data

No data available.

3.2.3 Other data

3.2.3.1 STUDY 1 - Acute neurotoxicity

Study reference: B.6.7.1, STUDY 3

Characteristics

Type of study	:	Acute neurotoxicity	Exposure	:	Single dose; 4 or 24 h semi-occluded
Year of execution	:	1990-1992	Doses	:	500, 1000, and 2000 mg/kg bw*

Test substance	:	MK-0243: L-656,748-038W, Lot 2 Purity: 94.2%	Vehicle	:	saline
Route	:	Dermal	GLP statement	:	Yes
Species	:	Rabbit NZW	Guideline	:	-
Group size	:	5 f	Acceptability	:	Acceptable
			LD50	:	> 2000 mg/kg bw*
			NOAEL		< 500 mg/kg bw*

* no factor was mentioned in the study report; Other studies with this batch used a conversion factor of 1.14, so it may be assumed that this was used for this study.

Study design

Six groups of 5 young adult female New Zealand White rabbits were administered L-656,748-038W (benzoate salt) in saline, applied once to the shaved, intact dorsal skin, under a semi-occlusive dressing, at dose levels of 500, 1000 and 2000 mg/kg bw for 4 or 24 hours. Collars prevented ingestion of the material, but time of collar removal was not specified. After exposure, the dressings were removed and the skin sites washed with water. Clinical signs and dermal irritancy were recorded daily for 14 days and body weights were recorded weekly. The animals were subjected to necropsy and examined post mortem, brain weights were recorded and samples of brain, spinal cord, peripheral nerve and gross lesions preserved, processed to haematoxylin & eosin stained sections and examined microscopically.

Results

4h exposure group

No mortality. Treatment-related systemic effects consisted of mydriasis and tremors. Mydriasis occurred in all animals in the 2000 mg/kg group and was first observed on day 3 and lasted up to day 5. Tremors were seen in only one animal of each of the 2000 and 500 mg/kg groups. No clinical signs were seen in animals of the 1000 mg/kg group. Transient dermal changes, consisting of very slight erythema were seen in one rabbit in each of the 500 and 2000 mg/kg dose groups on the first day of dosing. No differences were observed in body weights and brain weights between the groups and no treatment-related gross lesions were observed.

Test substance-related histomorphological alterations occurred in the brain, spinal cord and peripheral nerve and are presented in table 3.2.3.1-1. All dose groups were affected but the incidence tended to increase with dose level. The severity of effects was classified as very slight only.

24h exposure group

One high dose female exposed for 24 h showed marked signs of toxicity on day 6 (mydriasis, tremors, salivation/emesis, ataxia, decreased activity, a severe head tilt, and general weakness) and was killed on day 7. Mydriasis, usually beginning on day 3 as sluggish pupillary response was seen in every rabbit of all dose groups and persisted up to day 9 -11. Except for two animals at 500 mg/kg, all other animals showed tremors starting from day 3 onwards. The incidence generally increased with dose.

Lower mean body weights compared to pre-dose weights were observed on day 8, but persisted to day 14. Body weight losses of about 10% were seen in 2/5 rabbits in each of the low and mid dose groups and losses up to 15% in all 4 surviving rabbits of the high dose group. In the high dose group one rabbit showed slight erythema and oedema which were transient, and another rabbit showed well defined erythema starting from day 8 onwards. No skin effects were observed in the mid dose group. However, 3 animals of the low dose group showed slight to moderate erythema and oedema starting from day 4 or 7 up to the end of the study.

No differences were observed in brain weights between the groups and no treatment-related gross lesions were observed.

Test substance-related histomorphological alterations occurred in the brain, spinal cord and peripheral nerve and are presented in table 3.2.3.1-1. All dose groups were affected and the incidence and severity was increased compared to the 4-h exposure group. The effects were very slight in the low dose group, very slight to slight in the mid dose group, and very slight to moderate in the high dose group.

Table 3.2.3.1-1: inicidence of test substance-related microscopic findings (# of animals involved)

Dose (mg/kg bw)	4-hour exposure			24-hour exposure		
organ	**500 mg/kg bw**	**1000 mg/kg bw**	**2000 mg/kg bw**	**500 mg/kg bw**	**1000 mg/kg bw**	**2000 mg/kg bw**
Brain:						
- white matter degeneration	0	1	2	3	5	5
- neuronal degeneration	0	0	1	1	5	5
Spinal cord:						
- white matter degeneration.	3	0	1	3	4	5
- neuronal degen.	0	0	0	0	0	1
Peripheral nerve:						
- degeneration	1	2	4	4	5	5

Acceptability

Study design resembles OECD 424. Measurement of food consumption, functional observational battery and motor/locomotor activity was not performed. Since this study is only an acute exposure study, the deviations from the OECD 424 protocol are considered acceptable. In this study no control group was included.

Conclusions

A single dose of 500 mg/kg bw applied to the skin during 4 h only already induced clinical signs of neurotoxicity and substance-related morphological changes in spinal cord and peripheral nerves. The incidence and/or severity of the effects increased at higher dose levels and/or longer exposure duration, when also lesions in brain were observed. Skin irritation was also observed in several animals, but there was no dose relation.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 STUDY 1 - Acute toxicity study

Study reference: B.6.2.1.3, STUDY 1

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	4 h; nose only
Year of execution	:	2006	Dose	:	0, 0.239, 0.506, 1.049, 1.981 mg/L (achieved conc.)** MMAD: 2.7, 3.7, 3.8, and 2.9 resp.; GSD: 1.9, 2.6, 1.7, and 1.6 resp.
Test substance	:	MK 0244 G SNA6B019/milled (emamactin benzoate technical; purity 96.2%)	Vehicle	:	-
Route	:	Inhalation (aerosol)	GLP statement	:	yes
Species	:	Rat HsdRccHan:WIST strain	Guideline	:	OECD 403
Group size	:	5 m and/or f/group*	Acceptability	:	Acceptable
			LC50 rats	:	M: between 1.049 and 1.981 mg/L*** F: 0.663 mg/L

* in the low dose group (0.25 mg/L) only females were tested; in the mid dose group (1.0 mg/L) only males were tested.

** target dose levels were 0.25, 0.5, 1.0, and 2.0 mg/L

*** dose levels are expressed as benzoate salt. When expressed as base compound, the LC50 for males is between 0.920 and 1.738 mg/L and the LC50 for females is 0.582 mg/L.

Study design

The study design was based on OECD 403, but also on the 'up and down' procedure. The first exposure concentration tested was 2.0 mg/L and was based on previous studies (see below). Subsequent exposure concentrations were selected in a stepwise manner.

The test article is a white powder which was milled by the sponsor to reduce the particle size to a respirable range.

Results

Mortality:

Exposure concentration (mg/L)	**0.239**	**0.506**	**1.049**	**1.981**
Males	n.a.	0	0	4
Females	0	3	n.a.	5

n.a.: not applicable

Symptoms of toxicity: During exposure all animals of all exposure groups showed salivation, wet fur, associated with restraint and test substance staining around the snout. Reduced response to sound was observed in animals of the 0.506 mg/L dose group and above.

After exposure animals showed next to the symptoms mentioned above, decreased activity, hunched posture, piloerection, reduced response to sound, reduced righting reflex, and shaking for nearly all animals of the high dose group, some males of the 1.049 mg/L dose group, and several females of the 0.506 mg/L dose group. Male animals recovered within 9 days and females within 11 days.

Body weight: During the first week surviving males of the high dose group, several males of the 1.049 mg/L dose group, and the surviving females of the 0.506 mg/L dose group showed weight loss, but gained weight again in the second week.

Pathology: Only stained nostrils in the nasal cavity in one male of the high dose group was considered treatment-related. Other findings were not related to treatment.

Acceptability

The study is considered acceptable.

Conclusions

The acute 4-hour inhalation LC50 for emamectin benzoate salt in rats could be calculated for females and was 0.663 mg/L. The LC50 for males is between 1.049 and 1.981 mg/L. When expressed as base compound, the LC50 for females is 0.582 mg/L and the LC50 for males is between 0.920 and 1.738 mg/L.

Remark:

- No special attention has been given to findings in brain, spinal cord and/or sciatic nerve.

3.3.1.2 STUDY 2 - Acute toxicity study

Study reference: B.6.2.1.3, STUDY 2

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	4 h; nose only
Year of execution	:	1993	Dose	:	0, 0.24, 0.44, 2.12, 4.44 mg/L (achieved conc.)* MMAD:1.2, 4.1, 3.7, and 4.3 resp.; GSD: 3.0, 2.3, 2.0, and 2.1 resp.
Test substance	:	MK 0244 L656,748-052S 006 (Benzoate salt; purity 96.9%)	Vehicle	:	-
Route	:	Inhalation (aerosol)	GLP statement	:	yes
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	-
Group size	:	5 m/f	Acceptability	:	Acceptable
			LC50 rats	:	Between 2.12 and 4.44 mg/L (m/f)**

* the achieved concentrations (and also target and nominal concentrations) are expressed as benzoate salt.
** dose levels are expressed as benzoate salt. When expressed as base compound, the LC50 is between 1.83 and 3.83 mg/L.

Study design

The study resembles OECD 403. Deviations from protocol included some additional measurements: body weights were determined more frequent than prescribed, namely on days 2, 3, 4, 7 and 14; lung weights were determined; microscopic examination was performed on brain, spinal cord and sciatic nerve since changes in these tissues have been observed previously in rats.

Target concentrations were 0, 0.25, 0.50, 2.0 and 5.0 mg/L and the nominal concentrations 0, 0.77, 1.61, 5.51, and 11.71 mg/L. The test article is an off-white powder which was milled by the sponsor to reduce the particle size to a respirable range.

Results

Mortality: All animals of the high dose group died and 1f and 3m of the 2.12 mg/L dose group.

Symptoms of toxicity: Hypersalivation, trembling, lying on one side, ataxia, hypersensitivity to touch and gasping were noted in a few animals of the two low dose groups and in nearly all animals of the two high dose groups. These signs were observed between days 2 and 7.

Body weight: There were dose-related transient losses in the bodyweights of the majority of animals exposed to the test substance. In general, animals in the 0.24 and 0.44 mg/L groups had a slight decrease in bodyweight (less than 10%) between days 1 and 2, with the majority subsequently gaining bodyweight. In the two high dose groups, bodyweight losses were generally more severe. Between days 4 and 7, there were increases in the bodyweights of surviving animals in the 2.12 mg/L group.

Pathology: No treatment-related gross findings. However, histopathology was performed on brain, sciatic nerve and spinal cord. The number of animals found with microscopic findings in these tissues is summarized in the table below.

Exposure conc. (mg/L)	**0 m/f**	**0.24 m/f**	**0.44 m/f**	**2.12 m/f**	**4.44 m/f**
Brain:					
- neuron vacuolar degeneration	0/0	0/0	0/0	0/1	1/0
Spinal cord:					
- neuron vacuolar degeneration	0/0	0/0	0/0	0/1	0/0
- White matter degeneration	0/0	0/0	0/0	1/0	1/0
Sciatic nerve degeneration	0/0	1/4	5/5	2/4	0/0

Acceptability

The study is considered acceptable.

Conclusions

The acute 4-hour inhalation LC50 for emamectin benzoate hydrate in rats (m/f combined) is between 2.12 and 4.44 mg/L. When expressed as base compound, the LC50 is between 1.83 and 3.83 mg/L

Post mortem findings of neuronal vacuolar degeneration in the brain and spinal cord and/or sciatic nerve degeneration were evident in some animals from all groups, and thus a no effect level for these changes could not be established.

3.3.1.3 STUDY 3 - Acute toxicity study

Study reference: B.6.2.1.3, STUDY 3

Characteristics

Type of study	:	Acute toxicity study	Exposure	:	4 h; nose only
Year of execution	:	1993-4	Dose	:	0, 0.01, 0.05, 0.1 mg/L (achieved conc.)* MMAD: 3.2, 2.9, and 3.5 resp.; GSD: 2.0, 2.2, and 2.0 resp.
Test substance	:	MK 0244 L656,748-052S 006 (Benzoate salt; purity 96.9%)	Vehicle	:	-
Route	:	Inhalation (aerosol)	GLP statement	:	yes
Species	:	Rat Crl:CD(SD) BR strain	Guideline	:	-
Group size	:	5 m/f	Acceptability	:	Acceptable only for evaluation of effects in nerve tissue
			LC50 rats	:	Not determined.

* the achieved concentrations were equivalent to the target concentrations. The nominal concentrations were 0, 0.02, 0.08, and 0.17 mg/L. The concentrations are expressed as benzoate salt.

Study design

The study resembles OECD 403. However, the test was performed to establish a NOAEL for the histopathological changes observed in central and peripheral nerve tissue in the LC50 study mentioned above. The same additional measurements were performed.

Target and nominal concentrations were 0, 0.01, 0.05, 0.1 mg/L and the nominal concentrations 0, 0.02, 0.08, and 0.17 mg/L. The test article is an off-white powder which was milled by the sponsor to reduce the particle size to a respirable range.

Results

Mortality: No mortality.

Symptoms of toxicity: Increased activity was observed in some animals of the low dose group and in all animals of the higher dose groups between day 2 and 4 of observation.

Body weight: No effects.

Pathology: No treatment-related gross findings, and no microscopic changes in brain, spinal cord and sciatic nerve.

Acceptability

The study is considered acceptable as follow-up from the previous study for evaluation of the effects in the nerve tissue.

Conclusions

In a previous acute inhalation study (see study 2), exposure concentrations as low as 0.24 mg/L produced a lesion characterised primarily by very slight axonal swelling and degeneration in the sciatic nerves of exposed animals. No similar change was noted at any dose level in this study. Therefore, the high concentration group in this study (0.1 mg/L) is the NOAEL for this change. At this dose level, however, an increase in activity was noted during the first few days following exposure.

3.3.2 Human data

No data available.

3.3.3 Other data

No data available.

3.4 Skin corrosion/irritation

3.4.1 Animal data

3.4.1.1 STUDY 1 - skin irritation study

Study reference: B.6.2.2.1, STUDY 1

Characteristics

Type of study	:	skin irritation study	Exposure	:	4 h semi-occlusive
Year of execution	:	2006	Dose	:	500 mg
Test substance	:	Emamectin technical, benzoate salt Batch: SNA6B019 Purity: 96.2% (MK244)	Vehicle	:	destilled water
Route	:	dermal	GLP statement	:	yes
Species	:	Rabbit NZW	Guideline	:	OECD 404
Group size	:	1 m; 2f	Acceptability	:	Acceptable
			Effect	:	Not irritating to skin

Study design

The study is in accordance with OECD 404.

The test substance (0.5 g) was moistened with destilled water, applied to the test site and wrapped with a semi-occlusive dressing. Elizabethan collars were placed on the rabbits to prevent ingestion. Dermal changes were graded according to Draize.

Results

Irritation scores are presented in table 3.4.1.1.

Table 3.4.1.1 Individual skin irritation scores up to 72 h after application

	1 h f/m/f	**24 h f/m/f**	**48 h f/m/f**	**72 h f/m/f**
erythema	1/1/1	0/1/1	0/1/0	0/0/0
oedema	0/0/0	0/0/0	0/0/0	0/0/0

Acceptability

The study is acceptable.

Conclusions

Emamectin benzoate causes slight erythema when applied to the skin.

3.4.1.2 STUDY 2 - skin irritation study

Study reference: B.6.2.2.1, STUDY 2

Characteristics

Type of study	:	skin irritation study	Exposure	:	4 h semi-occlusive
Year of execution	:	1989-1992	Dose	:	500 mg
Test substance	:	MK 0243 L656,748 (4"-deoxy-4-epi-methylamino avermectine B1 benzoate); purity 96.2%)*	Vehicle	:	-
Route	:	dermal	GLP statement	:	yes
Species	:	Rabbit NZW	Guideline	:	In accordance with OECD 404
Group size	:	3 /sex	Acceptability	:	Acceptable
			Effect	:	Not irritating to skin.

Purity of substance: 91.1% B1a and 5.1% B1b.

Study design

The study is in accordance with OECD 404.

The test substance was applied to the test site and moistened with 0.5 mL saline and wrapped with a semi-occlusive dressing. Plastic collars were placed on the rabbits to prevent ingestion. Dermal changes were graded according to Draize. Observations were made at 30 to 60 minutes and 24, 48, 72, 144 and 168 hours after patch removal.

Results

No irritation was observed at any time point.

Acceptability

The study is acceptable.

Conclusions

Emamectin benzoate is not irritating.

3.4.2 Human data

No data available.

3.4.3 Other data

No data available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

3.5.1.1 STUDY 1 - eye irritation study

Study reference: B.6.2.2.2, STUDY 1

Characteristics

Type of study	:	eye irritation study	Exposure	:	Single instillation in conjunctival sac
Year of execution	:	2006	Dose	:	0.1 mL (weighing 60 mg)
Test substance	:	Emamectin technical, benzoate salt Batch: SNA6B019 Purity: 96.2% (MK244)	Vehicle	:	-
Route	:	ocular	GLP statement	:	yes
Species	:	Rabbit NZW	Guideline	:	OECD 405
Group size	:	2 m; 1f	Acceptability	:	Acceptable
			Effect	:	irritating to eyes

Study design

The study is in accordance with OECD 405.

Prior to each instillation, two drops of an ocular anaesthetic (tetracaine hydrochloride ophthalmic solution 0.5%) were placed into both the treated and control eye of each animal. Then the test substance was instilled in the test eye. Occasionally (marked with a * in the table) a fluorescein dye evaluation procedure was performed, to evaluate the extent or to verify the absence of corneal damage.

Ocular changes were graded according to Draize. The eyes were examined up to only 7 days after instillation (not up to 21 days as prescribed).

Results

The results up to day 7 are summarized in Table 3.5.1.1.

Table 3.5.1.1. Individual eye irritation scores in unwashed eyes up to 144 h after instillation

	1 h	24 h	48 h	72 h	Day 4	Day 7

	m/f/m	**m/f/m**	**m/f/m**	**m/f/m**	**m/f/m**	**m/f/m**
Corneal opacity	0/0/0	0*/1*/1*	0/0*/1*	0/0/1	0/0/1*	0/0/0*
Corneal area[#]	4/4/4	4/1/2	4/4/1	4/4/1	4/4/1	4/4/4
Iris	1/1/1	1/1/1	0/1/1	0/1/1	0/1/1	0/0/0
Conj. redness	2/2/2	2/2/2	1/2/2	0/2/2	0/2/2	0/0/0
Conj. chemosis	1/2/1	1/2/2	0/1/1	0/1/1	0/1/1	0/0/0
Conj. discharge	2/2/2	2/2/2	1/1/2	0/0/2	0/0/2	0/0/0

*: a fluorescein dye procedure was performed.
[#]: corneal area involved

Using fluorescein to evaluate opacity, it was found that only slight opacity was observed in 2 out of 3 rabbits.

Acceptability

The study is acceptable.

Conclusions

Signs of irritation were observed in the eyes of the rabbits. The mean scores for conjunctival redness and chemosis over the period 24 – 72 h are not greater than 2.5 and 2.0, respectively. The area of cornea involved was one quarter in one animal and was greater than a quarter but less than a half in the other animal at 24h.At 48h and 72h less than a quarter of the cornea was involved in the animal showing corneal opacity. Damage to the iris with score 1 was observed in all 3 animals up to 48 hours and still seen in 2 animals at 72 h.All signs of irritation (including effects on iris and corneal opacity) were reversible within the 7 day observation period of the study. After 7 days, no effects were observed in the corenea and this was seen in the total coreneal area involved (expressed as '4' in table 3.5.1.1 based on the Draize scores).

3.5.1.2 STUDY 2 - eye irritation study

Study reference: B.6.2.2.2, STUDY 2

Characteristics

Type of study	:	eye irritation study	Exposure	:	Single instillation in conjunctival sac
Year of execution	:	1989-1992	Dose	:	0.1 mL (weighing 28 mg)
Test substance	:	MK 0243 L656,748 (4"-deoxy-4-epi-methylamino avermectine B1 benzoate); purity 96.2%)*	Vehicle	:	-
Route	:	ocular	GLP statement	:	yes
Species	:	Rabbit NZW	Guideline	:	In accordance with OECD 405
Group size	:	3 /sex	Acceptability	:	Acceptable
			Effect	:	Severely irritating to the eyes

* Purity of substance: 91.1% B1a and 5.1% B1b.

Study design

The study is in accordance with OECD 405.

The test substance was grounded to a fine powder. Ocular changes were graded according to Draize. The eyes were examined up to 14 days after instillation, not up to 21 days.

Results

The results up to 144 h are summarized in Table 3.5.1.2-1.

Table 3.5.1.2-1. Individual eye irritation scores in unwashed eyes up to 144 h after instillation

	1 h m/f	**24 h m/f**	**48 h m/f**	**72 h m/f**	**144 h m/f**
Corneal opacity	0, 0, 0 / 0, 0, 0	0, 0, - / -, 0, 1	0, -, - / -, 0, 0	0, -, - / -, 0, 0	0, E, E / E, 0, 0
iris	0, 1, 1 / 0, 1, 1	1, 1, 1 / -, 1, 1	1, -, - / -, 1, 0	1, -, 1 / -, 1, 0	1, E, E / E, 1, 0
Conj. redness	2, 2, 2 / 2, 2, 2	3, 3, 3 / -, 3, 3	3, 3, 3/ -, 3, 1	3, 3, 3 / -, 3, 0	2, E, E / E, 2, 0
Conj. chemosis	2, 3, 2 / 3, 2, 3	3, 4, 4 / 4, 2, 1	3, 4, 4 / 4, 3, 0	2, 4, 4 / 4, 2, 0	1,E, E / E, 1, 0
Conj. discharge	3, 3, 3 / 3, 3, 3	3, 3, 2 / 3, 3, 0	3, 3, 3 / 3, 2, 0	3, 3, 2 / 3, 2, 0	2, E, E / E, 1, 0

-: indicates that the sign could not be read at that time point due to chemosis.
E: animal is euthanized following the 72 h reading, due to severe eye effects.

Because of severe ocular reactions, 3 rabbits (2m/1f) were euthanized following the 72 h reading.

Corneal anaesthesia was present in nearly all animals at 1 h and in one animal at 24 h. It was present in two of these animals up to 72h, but could not be checked in 3 other animals due to chemosis.

Of the surviving rabbits, one female still showed an effect on the iris up to day 7, conjunctival chemosis up to day 8 and conjunctival redness and discharge up to day 10, and the male rabbit showed conjunctival redness up to day 10 and discharge up to the end of the study (day 14).

Acceptability

The study is acceptable.

Conclusions

The mean scores for conjunctival redness and chemosis over the period 24 – 72 h are greater than 2.5 and 2.0, respectively. Furthermore, animals were sacrificed because of severe signs of irritation, and irritation was not reversible within the 14 day observation period of the study.

3.5.2 Human data

No data available.

3.5.3 Other data

No data available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data available.

3.6.2 Human data

No data available.

3.6.3 Other data

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 STUDY 1 - Skin sensitization study (GPMT)

Study reference: B.6.2.2.3, STUDY 1

Characteristics

Type of study	:	Skin sensitization study (GPMT)	Exposure	:	Intradermal and topical induction, topical challenge
Year of execution	:	1990-1992	Dose *	:	5% intradermal injection, 7.5% topical induction, 1.25% challenge
Test substance	:	MK 0244 L656,748-038W 002 purity > 95%	Vehicle	:	-
Route	:	dermal	GLP statement	:	yes
Species	:	Guinea pig (Hartley albino)	Guideline	:	In accordance with OECD 406
Group size	:	11 f (test group); 10 f (control)	Acceptability	:	Acceptable
			Effect	:	Not sensitizing

* - test concentrations were based on the results from screening tests: 5% intradermal was well tolerated but a 15% epicutaneous application evoked tremors in 2 out of 5 animals. In a 4-day exploratory irritation study, a concentration of 1.25% was not irritating, whereas a concentration of 2.5% was slightly irritating.

- A factor 1.14 was used in preparing the doses.

Study design

The study is in accordance with OECD 406. As the topical induction concentration was not irritating, the skin of the animals was treated with sodium lauryl sulphate before topical induction. Vehicle used was petrolatum. Challenge sites were read 24 and 48 h after removal of the patches. As positive skin reactions were observed a rechallenge was performed one week later on 3 control and 2 treated animals at a concentration of 0.5% and the sites were read after 24 and 48 h.

Results

No mortality and no clinical signs were observed.

Only after 48 h, slight erythema was observed in 2 test animals, but also in 3 control animals. Following re-challenge in these animals, only after 48 h slight erythema was observed in 1 test animal and in 1 control animal. No reactions were observed at the vehicle sites of any of the animals.

During histopathological examination, most animals of the control and treated group showed (very) slight acanthosis. This lesion was considered to be the result of non specific irritation due to occlusive application of the vehicle petrolatum.

Acceptability

The study is considered acceptable.

Conclusions

Under the conditions of the GPMT test, emamectin benzoate was not sensitizing.

3.7.1.2 STUDY 2 - Local lymph node assay

Study reference: B.6.2.2.3, STUDY 2

Characteristics

Type of study	:	Local lymph node assay	Exposure	:	topical on dorsal surface of the ears
Year of execution	:	2006	Dose *	:	0.5, 1, and 2.5% w/v MK244G
Test substance	:	Emamectin benzoate technical Batch: SNA6B019 Purity: 96.2% (MK244G)	Vehicle	:	dimethyl formamide
Route	:	dermal	GLP statement	:	yes
Species	:	Mouse CBA/Ca/Ola/Hsd strain	Guideline	:	OECD 429
Group size	:	4 f /group	Acceptability	:	Acceptable
			Effect	:	Not sensitizing

* - test concentrations were based on the results from screening tests in which animals were exposed to 3 repeat topical exposures of: 0.1, 0.5, 1, 2.5, and 5% w/v MK244G. Only the 5% w/v dose group showed signs of systemic toxicity.

Study design

The study is in accordance with OECD 429:

Approximately 25µl of a 0.5, 1 or 2.5 % w/v preparation of the test substance was applied to the dorsal surface of each ear. The procedure was repeated daily for 3 consecutive days. A control group was treated similarly with the vehicle, and a positive control group was treated with hexylcinnamaldehyde at 3 concentrations (5, 10, and 25% w/v).

Three days after the third application, all animals were injected with ^{3}H-methyl thymidine. Five hours later, the animals were humanely killed. The draining auricular lymph nodes from each ear were excised and pooled in PBS for each treatment group.

A single cell suspension was prepared and transferred to scintillation vials for ^{3}H-counting.

Animals were checked daily for clinical signs and body weights were recorded on days 1 and 6.

Results

There was no effect on body weight.

The stimulation index (SI) was 1.3, 1.1 and 2.1 for the low, mid, and high dose groups, respectively. Since the stimulation index was below 3, the test is considered negative.

The positive control group was positive (SI of 7.2) at the highest test concentration only.

Acceptability

The study is considered acceptable.

Conclusions

Under the conditions of the LLNA test, emamectin benzoate technical is considered not to be a skin sensitiser.

3.7.2 Human data

No data available.

3.7.3 Other data

No data available.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 STUDY 1 - microbial mutagenesis assay

Study reference: B.6.4.1, STUDY 1

Characteristics

Type of study	:	Ames test	Dose	:	953 µg/plate
Test substance	:	emamectin HCl salt (MK-0243), purity 92.8%	GLP	:	yes
Test system	:	S. typh. (TA 97a, TA 98, TA 100, TA 1535)	Guideline	:	resembles OECD 471
			Acceptability	:	acceptable
		E. coli (WP2, WP2 uvrA, WP2 UvrA pKK101)	Effect	:	negative

Study design

The study design resembles OECD 471. Emamectin was tested in the microbial mutagenesis assay using *Salmonella typhimurium* tester strains (TA1535, TA97a, TA98 and TA100) and *Escherichia coli* tester strains (WP2, WP2 uvrA, and WP2 uvrA pKM101) with and without metabolic activation by liver microsomal enzymes from rats pre-treated with xenobiootics. DMSO was included as a negative control. Appropriate positive controls were included. In fact, for the salmonella tester strains and *E. coli* strains WP2 uvrA and WP2 uvrA pKM101 2-aminoanthracene was used and for *E. coli* WP2 this was hydrazine sulfate.

Results

MK-0243 (purity 92.8%) did not induce ≥ 2-fold increase in revertant colonies either with or without metabolic activation. A high dose of 953 µg/plate was used in this assay since previous experience with emamectin compounds indicated that this is the approximate level where bacterial toxicity was seen. No precipitation was seen at a dose of 953 µg/L. The positive controls 2-aminoanthracene and hydrazine sulfate induced the expected increases in revertant colonies in all strains, in the presence of metabolic activation.

Table 3.8.1.1-1 Results from bacterial reverse mutation assay.

Indicator cells	Endpoint	Result -act	Result +act	Activation tissue	Activation inducer	Dose range [1]	Reference
S. typh. TA 97a TA 98 TA 100 TA 1535 *E. coli* WP2 WP2 uvrA WP2 uvrA pKK101	 point mut. point mut. point mut. Point mut. point mut point mut point mut	 - - - - - - -	 - - - - - - -	rat liver	Aroclor 1254	0, 2, 8, 19, 211, 953 µg/plate solvent: DMSO	Lankas, 1992b
test substance	MK-0243, batch L-656,748-010V003, HCl salt, purity: avermectine B1a is 92.8% by HPLC, avermectine B1b is 4.1% by HPLC, exposure duration 48h.						
GLP Guideline	Yes EPA data requirement subdivision F, Series 84-2. Test design resembles OECD 471						

1 The highest dose is based on bactericidal activity observed in previous tests. In the present study toxicity (inhibition of growth) was observed in most strains at 953 µg/plate.

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, emamectin HCl salt (MK-0243) did not induce point mutations in *S. typhimurium* or *E. coli*.

3.8.1.2 STUDY 2 - mutagenicity test using V-79 Chinese hamster lung fibroblasts

Study reference: B.6.4.1, STUDY 2

Characteristics

Type of study	:	Chromosome aberrations	Dose	:	0, 0.005- 0.06 mM with S9, 0, 0.001-0.01 mM without S9
Test substance	:	MK-0244, batch L-656,748-010V003, HCl salt, purity 96.9%	GLP	:	yes
Test system	:	Chines hamster V79 cells	Guideline	:	resembles OECD 476
			Acceptability	:	acceptable
			Effect	:	negative

Study design

Test design resembles OECD 476 and was performed to detrrmine the potential of emamectin (purity 96.6%) to mutagenicity in V-79 Chinese hamster lung fibroblasts. The test was performed with and without metabolic activation. DMSO was used as a negative control. A range finding cytotoxicity test was performed prior to the mutagenicity assay and cytotoxicity was assessed as reductions in total cell number or monolayer confluence, abnormal cell morphology and inhibition of cell division. Different concentrations of emamectin were tested (0.003; 0.01; 0.03; 0.1; 0.3; 1.0; 3.0 and 10.0 mM). Duplicate samples of each compound concentration were tested. In the main test V-79 Chinese hamster lung fibroblasts were exposed to emamectin and appropriate controls for 3 h at concentrations of emamectin rangeing between 0.005-0.06 mM with metabolic activation and 0.005-0.04 mM without metabolic activation. The positive controls used were 3-methylcholanthrene for incubations with metabolic activation and methylnitrosourea for incubations without metabolic activation. In the test performed without metabolic activation, cell survival was relatively low and therefore treatment in the presence of metabolic activation was repeated using cocnentrations of emamectin of 0.001-0.01 mM. Dose selection was based on either the toxicity or the solubility of the test compound. Mutations at the hpt locus were measured as resistance to 6-thioguanine after 7, 9 or 12 days.

Results

Emamectin did not induce an increased resistance to 6-thioguanine under the test condition in the absence or presence of metabolic activation. A positive dose response relation was lacking. A significant increase in aberration was induced by the positive controls.

Relative survival ranged from 70 - 2% with S-9 and from 101 - 4% without S-9 with ascending concentration.

Table 3.8.1.2 Results from a gene mutation test with MK-0244 in mammalian cells, *in vitro*

Indicator cells	Endpoint	Result -act	Result +act	Activation tissue	Activation inducer	Dose range
Chinese hamster V79	gene mutation	-	-	rat liver	Aroclor 1254	0, 0.005- 0.06 mM with S9, 0, 0.001-0.01 mM without S9 solvent: DMSO
test substance	MK-0243, batch L-656,748-010V003, HCl salt, purity 96.9% by HPLC, exposure duration 3h.					
GLP	Yes					
Guideline	EPA data requirement subdivision F, Series 84-2. Test design resembles OECD 476					

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, emamectin HCL salt (MK-0243) did not induce gene mutations in mammalian cells.

3.8.1.3 STUDY 3 - chromosome aberrations in Chinese hamster ovary (CHO) cells

Study reference: B.6.4.1, STUDY 3

Characteristics

Type of study	:	Chromosome aberrations	Dose	:	0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µM
Test substance	:	MK-0244, batch L-656,748-052S002, purity avermectine B1a 92.5%, purity avermectine B1b 5.3%.	GLP	:	yes
Test system	:	Chines hamster oveary cells (CHO-WBL)	Guideline	:	resembles OECD 473
			Acceptability	:	acceptable
			Effect	:	negative

Study design

Study design resembles OECD 473 and was performed to determine if emamectin has the potential to cause chromosome aberrations in Chinese hamster ovary (CHO) cells. The test was performed in the presence and absence of metabolic activation. Prior to the main test a range finding study was performed in order to select appropriate doses for the main test. In the main test, the cells were treated for a period of 3 hours with emamectin or a control and fixation time was 20 hours. Different concentrations rangeing between 6-8µM and 2-4µM were tested, in the absence and presence of 10% fetal bovine serum, respectively. Appropriate positive controls were included (cyclophosphamide and mitomycin for incubations with and without metabolic activation, respectively).

The test was performed twice, since in the first test a chemical assay of solutions suggested the presence of a contaminant in the DMSO which appeared to react with MK-0244. Therefore the first test was considered invalid.

Results

In the second test no statistically significant increased incidence in chromosome aberrations was observed for dose levels up to 7 µM (with S-9) or 6 µM (without S-9). Moreover, a dose-related response was not observed. Marked inhibition of growth was seen at doses ≥ 8 µM (with S-9) and ≥ 7 µM (without S-9). Significant increases in chromosome aberrations were observed in incubations performed with the positive controls.

Table 3.8.1.3-1 Results from a chromosome aberration test with MK-0244 in mammalian cells, *in vitro*

Indicator cells	Endpoint	Result -act	Result +act	Activation tissue	Activation inducer	Dose range [1]	
Chinese Hamster Ovary cells (CHO-WBL).	chromo-some aberrations	-	-	rat liver	Beta-naphthoflavone and phenobarbital	0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µM. solvent:DMSO	
test substance	MK-0244, batch L-656,748-052S002, purity of avermectine B1a is 92.5% by HPLC, purity of avermectine B1b is 5.3% by HPLC. Exposure duration 3h with subsequent reincubation for17h.						
GLP	Yes						

Guideline	EPA data requirement subdivision F, Series 84-2. Study design resembles OECD 473

[1] In a range-finding test, toxicity was observed from 10 µM onwards, with and without S-9. In the range finding test mitotic suppression was seen at 10 µM with S-9 and at 5 and 10 µM without S-9.

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, emamectin benzoate hydrate salt (MK-0244) did not induce chromosome aberrations in mammalian cells (Chinese hamster ovary cells) up to doses that caused marked growth reduction.

3.8.1.4 STUDY 4 - single- and double-strand DNA breaks

Study reference: B.6.4.1, STUDY 4

Characteristics

Type of study	:	DNA strand breaks	Dose	:	0.003- 0.02 mM
Test substance	:	MK-0243, batch L-656,748-010V003, HCl salt, purity 92.8%, Note: In another study (STUDY 1) by the same study author the same batch was used. In that study purity was reported as: avermectine B1a 92.8%, avermectine B1b 4.1%	GLP	:	yes
Test system	:	rat hepatocytes in an in vitro alkaline elution/rat hepatocyte assay	Guideline	:	-
			Acceptability	:	acceptable
			Effect	:	negative

Study design

The potential of MK-0243 to induce single- and double-strand DNA breaks and cytotoxicity was tested in rat hepatocytes in an *in vitro* alkaline elution/rat hepatocyte assay. In this test an aliquot of cell suspension (1x 10^6 total cells) is loaded on a 2.0 µm polycarbonate filter and lysed. Subsequently a solution of tetrapropyl ammonium hydroxide, pH 12.1 is added to the DNA on the filter and three fractions of 3h elutions are collected. The DNA from each fraction is trapped on a 0.2 µm polycarbonate filter and the amount of DNA on each filter is measured. Elution slopes three times greater than controls are considered to indicate meaningful increases in DNA strand breaks. Since cytotoxicity itself can induce DNA strand breaks, the increase in elution slope should not be correlated with increased toxicity (<70% cell viability).

Isolated rat hepatocytes were incubated for 3h with MK-0243 at concentrations of 0 (1% DMSO), 0.003, 0.006, 0.010 and 0.020 mM. Concentrations were based on cytotoxicity induced by MK-0243 in a range finding study. After 3 h of incubation cells were harvested and tested for cytotoxicity by trypan blue exclusion, and for DNA strand breaks by the alkaline elution assay. Aflatoxin B1 was used as positive control.

Results

In a first test, MK-0243 at 0.01 mM induced a 2.86-fold increase in elution slope, with 76% relative cell viability. At 0.02 mM relative cell viability was only 4%. Since the increase in elution slope at 0.01 mM was

close to the 3-fold increase criterium a second test was performed with MK-0243 at concentrations of 0.006, 0.008, 0.010, 0.012 and 0.014 mM. In this test there was a less than 3-fold increase in the elution slope at concentrations up to and including 0.010 mM. At 0.012 and 0.014 mM the relative survival was less than 70%.

Aflatoxin B1 induced a 10- and 18-fold increase in the elution slope in the first and second test, respectively.

Under the test conditions emamectin HCl salt (MK-0243) did not induce DNA strand breaks in primary rat hepatocytes.

Table 3.8.1.4-1 Results from alkaline elution/rat hepatocyte assay with MK-0243

Indicator cells	Endpoint	Result	Dose range
Rat primary hepatocytes (male Crl:CD (SD) BR rats)	DNA strand breaks	-	0.003- 0.02 mM, solvent: DMSO
test substance	MK-0243, batch L-656,748-010V003, HCl salt, purity 92.8% by HPLC, exposure duration 3h. Note: In another study (STUDY 1) by the same study author the same batch was used. In that study purity was reported as: avermectine B1a 92.8% by HPLC, avermectine B1b 4.1% by HPLC		
GLP	Yes		
Guideline	EPA data requirement subdivision F, Series 84-2. Test design unknown		

Acceptability

There is no official guideline for this kind of study. The study is considered supplementary.

Conclusions

Under the test conditions emamectin HCl salt (MK-0243) did not induce DNA strand breaks in primary rat hepatocytes.

3.8.2 Animal data

3.8.2.1 STUDY 1 - *in vivo* chromosome aberration test in mice

Study reference: B.6.4.2, STUDY 1

Characteristics

Type of study	:	Chromosome aberrations	Dose	:	single oral exposure 0, 8, 26, 80 mg/kg bw
Test substance	:	MK-0244, batch L-656,748-052S002, purity 95.9%	GLP	:	yes
Species/ organism	:	Mouse (Crl:CD-1 (ICR) BR, 5males/ dose	Guideline	:	-
			Acceptability	:	acceptable
			Effect	:	negative

Study design

Male mice received by gavage a single dose of MK-0244 in 0.5% aqueous methylcellulose in doses of 0, 8, 26 or 80 mg/kg bw (5 males/ dose). Bone marrow was harvested at 6, 12 and 48 hr after treatment and chromosome preparations were made. At each time point 12, 8, 8, and 10 animals of respectively the control, low, mid and high dose group were killed. Mitomycin G was used as positive control.

Results

Clinical signs (tremors, erect tails, decreased activity, ptosis, bradypnoea and hypothermia) were observed in the high dose group throughout the study. One animal in the high dose group died. In the mid-dose group all animals had ptosis 6h after administration. At 24 and 48h occasionally ptosis and tremors were observed in this group. No clinical signs were observed in the low dose group. No statistically significant increase in chromosome aberrations was observed in the MK-0244-treated mice. Mitomycin G induced highly significant increases in chromosome aberrations.

Table 3.8.2.1-1 Results from an *in vivo* chromosome aberration test in mice with MK-0244

Species	Endpoint	Result	Dose range
Mouse (Crl:CD-1 (ICR) BR, males	Chromosome aberrations	-	single oral exposure 0, 8, 26, 80 mg/kg bw (expressed as free base) vehicle: 0.5% aqueous methylcellulose
test substance	MK-0244, batch L-656,748-052S002, purity 95.9% by HPLC.		
GLP	Yes		
Guideline	EPA data requirement subdivision F, Series 84-2. Design resembles OECD 475		

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, emamectin benzoate hydrate (MK-0244) did not induce chromosome aberrations in mouse bone marrow cells.

3.8.3 Human data

No data available.

3.8.4 Other data

No data available.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 STUDY 1 - dietary carcinogenicity/toxicity study in rats

Study reference: B.6.5.1, STUDY 1

Characteristics

Type of study	:	dietary carcinogenicity/toxicity study in rats	Exposure	:	Repeated by diet, 104 weeks
Year of execution	:	1991-1993	Doses[b]	:	0, 0, 0.25, 1.0, and 5.0[c]/ 2.5[d] mg/kg bw/day
Test substance	:	MK-0244 (L-656,748-052S, technical; purity 95.9% at initiation, 97.4 to 98.6% weeks 10, 41, 60, 82 and 105)[a]	Vehicle	:	
Route	:	oral	GLP statement	:	yes
Species	:	Rat, Sprague-Dawley Crl:CD(SD)BR	Guideline	:	No guideline, but comparable to OECD 453
Group size	:	Test substance: 75/sex/dose Controls: 130/sex	Acceptability NOAEL	: :	acceptable 0.25 mg/kg bw/day (general tox). No carcinogenicity observed

a: the stability of the test compound was not reported (but was determined to be satisfactory in this study by the notifier).

b: the dose of emamectin benzoate hydrate salt were calculated as base compound by using a factor of 1.15 (based on the stoichiometry of water in the MK-0244 crystal structure) (note of the notifier)

c: f: weeks 1-9 and m: weeks 1-5

d: f: weeks 10-104 and m: weeks 6-104

Study design

Rats (75/sex/dose) were given 0.25, 1.0 or 5.0 mg/kg bw/day MK-0244 for 104 weeks in their diet (doses based on 3- and 13-weeks range-finding studies with rats, see DAR B.6.3.1. study 1 and DAR B.6.3.3. study 1). A control group (130/sex) was included. Due to (unacceptable) weight loss and tremors in males at 5.0 mg/kg bw/day in another study of MK-0244 in week 9 (14 week neurotoxicity study in rats, see DAR B.6.7.2 study 4), the dose of 5.0 mg/kg bw/day was reduced to 2.5 mg/kg bw/day for males starting week 6. For female rats in the 5.0 mg/kg bw/day group the dose was reduced to 2.5 mg/kg bw/day in week 10 (because of observed tremors in females at 5.0 mg/kg bw/day in week 11 of abovementioned rat neurotoxicity study (see DAR B.6.7.2 study 4). The study performance was comparable to OECD guideline 453. Statistical evaluation was done using the trend test for body weight (survivors only) and mortality.

Results

Table 3.9.1.1-1 Results from a 105 week oral toxicity study in rats

Dose (mg/kg bw/day)	**0**		**0.25**		**1.0**		**5.0/ 2.5**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No treatment-related increase								
Clinical signs -unkempt -urine staining -lethargic -footsore hind	 23% 7% 29%	 9% 29% 	 	 	 	 	 39% 16% 64%	 17% 40% 	
Body weight gain (g)	533	361	573	329	540*	371*	430*	441*	
Food consumption	No toxicologically relevant effects								
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology (week 105) - segmented neutrophils (cells/mm^3) -eosinophils (cells/mm^3)	 4518 	 39	 4822 	 32	 4632 	 35	 6555 	 306	

Clinical Chemistry (week 105)		50		52		47		92	
-ALT (u/L)	449	439	486	302	319	355	266	547	
-CK (u/L)	4.9	4.4	5.2	5.0	5.4	5.3	5.8	6.0	m,
-potassium (meq/L)		6.5		6.8		6.9		7.4	f
-phosporus (meq/L)	308	168	176	194	167	383	152	954	f
-triglycerides, week 79 (mg/dL)									m, f
Urinalysis	No toxicologically relevant effects								
Organ weights									
-kidney (% bw)	0.71		0.77		0.90		0.93		m
-liver (% bw)	2.36		2.66		3.00		3.54		m
-testes (% bw)	0.48		0.45		0.43		0.58		
Pathology									
macroscopy									
-foot sores (plantar granulomas)	29%		12%		29%		64%		
-tail nodule	13%						27%		
microscopy									
-brain, neuron vacuolar degeneration	0	0	0	0	0	0	27/28	12/23	
-spinal cord, neuron vacuolar degeneration	0	0	0	0	0	0	15/28	1/23	
-kidney chronic nephritis		53%		63%		63%		80%	
-urinary bladder, chronic proliverative cystes	7%		7%		7%		17%		

dr = dose related; * $p \leq 0.05$

In contrast to the 13-week and 52-week studies with rats, no clinical signs of neurotoxicity were observed in this study. Increased body weight gain in females of the mid (10-20%) and high (10-28%) dose group was observed after the first weeks of the study until termination. In high dosed males loss of body weight was observed from week 46 of the study, resulting in a statistically significant lower terminal average body weight than control males. An increased number of segmented neutrophils in high dose males in weeks 79 and 105 were observed. At all dose levels, triglyceride levels were decreased in males; however, this was likely due to high control values, which were partly higher than historical control values. In females, a dose-related increase in triglyceride levels was observed, which is considered toxicologically relevant at and above 1.0 mg/kg bw/day. These effects on blood triglyceride levels were most prominent at weeks 52 and 79, but also apparent at week 105. At the mid and high dose, males had increased relative liver and kidney weights, which were not accompanied by histopathological changes.

Terminal microscopic examination showed vacuolar degeneration in neurons of brain and spinal cord of high dose animals. These observations were most prominent in males. These changes were also observed in early death high dose animals. In high dose females a higher incidence of chronic nephritis was observed. In high dose males more chronic proliferative cystes were observed in the urinary bladder. In early death animals higher incidences of prostate chronic inflammation (high dose males), heart chronic focal myocarditis (high dose females), Harderian gland chronic inflammation (mid and high dose females) and skeletal muscle atrophy (high dose females) were observed. There was no treatment-related increase in tumours.

Table 3.9.1.1-2 Tumor incidences from a 105 week oral toxicity study in rats, female rats

	Control (0)	**0.25 mg/kg bw/day**	**1.00 mg/kg bw/day**	**5.00/ 2.50 mg/ kg bw/day**		
No. of animals tested	**129**	**75**	**75**	**75**	**Trend p-value (before adjustment)**	**Trend adjusted for multiple tumor sites**

1	Skin – lipoma	4 (3%)	1 (1%)	0 (0%)	5 (7%)	0.033	NSd (p>0.500)
2	Skin - papiloma	0 (0%)	1 (1%)	0 (0%)	1 (1%)	0.214	NS
3	Skin - fibroma	3 (2%)	0 (0%)	0 (0%)	0 (0%)	0.044N	NSe (P>0.500N)
4	Large intestine – polyp	0 (0%)	0 (0%)	2 (3%)	1 (1%)	0.089	NS
5	Brain – benign glandular cell tumor	3 (2%)	0 (0%)	5 (7%)	3 (4%)	0.098	NS
6	Brain – malignant glioma	2 (2%)	0 (0%)	1 (1%)	0 (0%)	0.229N	NS
7	Mammary gland - adenocarcinoma	23 (18%)	14 (19%)	14 (19%)	18 (24%)	0.105	NS
8	Mammary gland – adenoma	8 (6%)	4 (5%)	4 (5%)	7 (9%)	0.113	NS
9	Mammary gland fibroadenoma	70 (54%)	45 (60%)	44 (59%)	36 (48%)	0.075N	NS
10	Pancreas – acinus, adenoma	1 (1%)	0 (0%)	1 (1%)	1 (1%)	0.257	NS
11	Pancreas – islet, carcinoma	6 (5%)	0 (0%)	1 (1%)	3 (4%)	0.266	NS
12	Pancreas – islet, adenoma	17 (13%)	8 (11%)	5 (7%)	10 (13%)	0.334	NS
13	Adrenal – cortex, adenoma	4 (3%)	1 (1%)	1 (1%)	3 (4%)	0.328	NS
14	Adrenal – benign pheochiromgytoma	4 (3%)	4 (5%)	3 (4%)	0 (0%)	0.061N	NS
15	Adrenal – cortex, carcinoma	2 (2%)	1 (1%)	1 (1%)	0 (0%)	0.228N	NS
16	Parathyroid – adenoma	1 (1%)	0 (0%)	0 (0%)	1 (1%)	0.348	NS
17	Kidney – tubular adenoma	1 (1%)	1 (1%)	1 (1%)	1 (1%)	0.379	NS
18	Histologic sarcoma	0 (0%)	2 (3%)	0 (0%)	1 (1%)	0.385	NS
19	Lymphoma	3 (2%)	1 (1%)	1 (1%)	0 (0%)	0.144N	NS
20	Ear – zymbal’s gland, carcinoma	1 (1%)	0 (0%)	0 (0%)	1 (1%)	0.447	NS
21	Thyroid – follicular cell, adenoma	2 (2%)	0 (0%)	0 (0%)	1 (1%)	>0.500	NS
22	Thyroid – parafollicular cell, adenoma	12 (9%)	6 (8%)	8 (11%)	4 (5%)	0.169N	NS
23	Thyroid – parafollicular cell, carcinoma	1 (1%)	1 (1%)	2 (3%)	0 (0%)	0.313N	NS
24	Follicular cell, carcinoma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	0.477N	NS
25	Uterus – polyp	11 (9%)	1 (1%)	2 (3%)	4 (5%)	0.106N	NS
26	Uterus – endometrium adenoma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	0.415N	NS
27	Uterus – fibroma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	>0.500N	NS
28	Pituitary - adenoma	104 (81%)	66 (88%)	57 (76%)	60 (80%)	0.186N	NS
29	Liver – hepatocellular carcinoma	1 (1%)	1 (1%)	0 (0%)	0 (0%)	0.235N	NS
30	Liver – hepatocellular adenoma	3 (2%)	0 (0%)	1 (1%)	1 (1%)	0.370N	NS
31	Stomach – nonglandular mucosa, papilloma	1 (1%)	1 (1%)	0 (0%)	0 (0%)	0.331N	NS
32	Liver - adenocarcinoma	0 (0%)	2 (3%)	0 (0%)	0 (0%)	0.334N	NS
33	Vagina - polyp	1 (1%)	0 (0%)	2 (3%)	0 (0%)	0.336N	NS

D significant level assessed based on the number of tumor sites, k1-25, for which a more extreme positive p-value should have been observed

E as in D (above), but for more extreme negative trend p-values with k1=21

NS not statistically significant, P> 0.05

N indicate a reverse carcinogenic effect

Table 3.9.1.1-2 Tumor incidences from a 105 week oral toxicity study in rats, male rats

	Control	**0.25**	**1.00**	**5.00/**		

		(0)	**mg/kg bw/day**	**mg/kg bw/day**	**2.50 mg/ kg bw/day**		
No. of animals tested		**130**	**75**	**75**	**75**	**Trend p-value (before adjustment)**	**Trend adjusted for multiple tumor sites**
1	Skin – lipoma	1 (1%)	2 (3%)	4 (5%0	5 (7%)	0.010	NSd (p = 0.222)
2	Skin - papiloma	2 (2%)	1 (1%)	3 (4%)	3 (4%)	0.119	NS
3	Skin - fibroma	3 (2%)	0 (0%)	4 (5%)	3 (4%)	0.146	NS
4	Skin sebaceous adenoma	0 (0%)	1 (1%)	1 (1%)	0 (0%)	>0.500	NS
5	Skin – benign keratocanthoma	5 (4%)	3 (4%)	5 (7%)	1 (1%)	0.150N	NS
6	Small intestine - adenocarcinoma	0 (0%)	0 (0%)	1 (1%)	2 (3%)	0.049	NSe (P>0.500)
7	Small intestine - polyp	1 (1%)	0 (0%)	0 (0%)	1 (1%)	0.241	NS
8	Testis – benign interstitial cell tumor	6 (5%)	3 (4%)	3 (4%)	7 (9%)	0.061	NS
9	Testis – benign mesothelioma	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0.094N	NS
10	Thyroid – parafollicular cell, carcinoma	1 (1%)	0 (0%)	0 (0%)	2 (3%)	0.101	NS
11	Thyroid – parafollicular cell, adenoma	14 (11%)	5 (7%)	4 (5%)	5 (7%)	0.089N	NS
12	Thyroid – follicular cell, adenoma	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0.150N	NS
13	Large intestine - polyp	0 (0%)	0 (0%)	1 (1%)	1 (1%)	0.140	NS
14	Ear – zymbal’s gland, adenoma	0 (0%)	1 (1%)	0 (0%)	1 (1%)	0.176	NS
15	Kidney – tubular adenoma	1 (1%)	0 (0%)	1 (1%)	1 (1%)	0.302	NS
16	Kidney – liposarcoma	2 (2%)	0 (0%)	4 (5%)	0 (0%)	0.421	NS
17	Kidney – lipoma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	>0.500N	NS
18	Pituitary - adenoma	83 (64%)	48 (64%)	35 (47%)	46 (61%)	0.324	NS
19	Pancreas – acinus, adenoma	1 (1%)	1 (1%)	1 (1%)	1 (1%)	0.474	NS
20	Pancreas – ilset, adenoma	23 (18%)	14 (19%)	13 (17%)	9 (12%)	0.076N	NS
21	Pancreas – islet, carcinoma	3 (2%)	1 (1%)	1 (1%)	1 (1%)	0.395N	NS
22	Mammary gland - fibroadenoma	4 (3%)	1 (1%)	2 (3%)	2 (3%)	0.482	NS
23	Parathyroid - adenoma	1 (1%)	0 (0%)	0 (0%)	1 (1%)	0.484	NS
24	Liver – hepatocellular adenoma	2 (2%)	1 (1%)	0 (0%)	1 (1%)	>0.500	NS
25	Liver – hepatocellular carcinoma	5 (4%)	7 (9%)	4 (5%)	3 (4%)	>0.500	NS
26	Brain – benign granular cell tumor	2 (2%)	3 (4%)	0 (0%)	0 (0%)	0.076N	NS
27	Brain – malignant glioma	1 (1%)	2 (3%)	0 (0%)	0 (0%)	0.231N	NS
28	Adrenal malignant pheochromocytoma	3 (2%)	3 (4%)	0 (0%)	1 (1%)	0.107N	NS
29	Adrenal – cortex, adenoma	0 (0%)	3 (4%)	0 (0%)	0 (0%)	0.234N	NS
30	Adrenal – benign pheochromocytoma	12 (9%)	9 (12%)	6 (8%)	6 (8%)	0.323N	NS
31	Histiocytic sarcoma	2 (2%)	1 (1%)	0 (0%)	0 (0%)	0.168N	NS
32	Myeloid leukemia	1 (1%)	1 (1%)	0 (0%)	0 (0%)	0.272N	NS
33	Fibrous malignant histiocytoma	0 (0%)	2 (3%)	0 (0%)	0 (0%)	0.321N	NS
34	lymphoma	3 (2%)	6 (8%)	1 (1%)	3 (4%)	0.369N	NS
35	Spinal cord – malignant glioma	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0.171N	NS
36	Spleen - hemangioma	1 (1%)	1 (1%)	0 (0%)	0 (0%)	0.336N	NS
37	Lymph node - hemangioma	1 (1%)	1 (1%)	1 (1%)	0 (0%)	0.399N	NS
38	Prostate - adenocarcinoma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	0.439N	NS
39	Thymus – benign thymoma	1 (1%)	0 (0%)	1 (1%)	0 (0%)	>0.500N	NS

D significant level assessed based on the number of tumor sites, k1-25, for which a more extreme positive p-value should have been observed

E as in D (above), but for more extreme negative trend p-values with k1=21

NS not statistically significant, P> 0.05

N indicate a reverse carcinogenic effect

Acceptability

The study is considered acceptable.

Conclusions

In high dose animals several parameters were changed, the most prominent effect being vacuolar degeneration of neurons in brain and spinal cord and effects on bodyweight gain. Based on effects observed on female blood triglyceride levels and on male relative weights of kidney and liver at and above 1.0 mg/kg bw/day, the NOAEL in this study is 0.25 mg/kg bw/day. In this study with rats, no substance-related increase in tumours was observed.

3.9.1.2 STUDY 2 - Carcinogenicity study in mice

Study reference: B.6.5.1, STUDY 2

Characteristics

Type of study	:	Carcinogenicity study in mice	Exposure	:	Repeated by diet, 79 weeks
Year of execution	:	1991-1992	Doses[a]	:	m: 0, 0, 0.5, 2.5 and 12.5/7.5/5 mg/kg bw/day f: 0, 0, 0.5, 2.5 and 12.5/7.5 mg/kg bw/day
Test substance	:	MK-0244 (L-656,748-052S (Lot #2); purity 97.%)	Vehicle	:	-
Route	:	Oral	GLP statement	:	Yes, with exceptions
Species	:	Mouse, Crl:CD-1	Guideline	:	no guideline, but comparable to OECD 451
Group size	:	50/sex/group	Acceptability	:	acceptable
			NOAEL	:	2.5 mg/kg bw/day (general tox). No carcinogenicity observed

a: since the compound was provided as benzoate salt and to account for the stoichiometry of water in the MK-0244 crystal structure doses of the compound were calculated as base compound by using a factor of 1.15 (except week 1 in which 1.16 was used)

Study design

The doses for this study were based upon the results of a previously conducted 13-week mice dietary toxicity study.

Mice (50/sex/dose) were given 0.5, 2.5 or 12.5 mg/kg bw/day MK-0244 for 79 weeks in their diet; two control groups were included. An additional 15/sex/dose (one control group) were placed on study for haematological examination only after 1 year. Due to an increased incidence in mortality, decreased body weights and tremors, the high dose for males was reduced to 7.5 mg/kg bw/day during week 9, and further reduced to 5.0 mg/kg bw/day from week 31 until termination (due to continued decreased weight gain/ body weight loss). For high dose female mice the dose was reduced to 7.5 mg/kg bw/day in week 48 (because of decreased weight gain). The study was initially scheduled for a duration of 93 weeks, but due to increased mortality at the high dose level, the study was terminated in week 79. The study performance was

comparable to OECD guideline 451.Statistical analysis was done using a trend test for body weight (survivors only) and mortality.

Results

Table 3.9.1.2-1 Results from a 79 week oral carcinogenicity study with mice

Dose (mg/kg bw/day)	**0**		**0.5**		**2.5**		**12.5/7.5/5.0**	**12.5/7.5**	**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
-Mortality (weeks 3-11) -total -Trend p-value	34%	25%	21%	22%	21% 0.188	26% 0.422	7 68% <0.001	 60% <0.001	
Clinical signs -tremor -vocalization -fine fasciculating tremor of forequarter/forelimbs -skin lesions (cracking, exudate formation, hair loss, scabbing)	49%	6%	48%	12%	46%	18%	3/50 5/50 35/50 84%	 5/50 45/50 74%	f
Mean body weight gain (g) Final body weight (g)	15.8 45.3	15.8 37.7	16.9 44.2	17.5 37.8	16.7 43.2	15.5 36.8	5.1* 33.6 (-26%)	7.6* 28.5 (-24%)	
Food consumption	No toxicologically relevant effect								
Water consumption	Not determined								
Ophthalmoscopy -blepharitis and corneal scarring							11/50	9/50	
Haematology (week 79) -segm. neutrophils (%) -lymphocytes (%) -ery's (million/mm^3) -Hb (g/100 mL) -MCV (cubic microns) -MCHC (g/dL) -leucocytes (1000/mm^3) -lymphocytes (cells/mm^3)	 7.8 12.3 45.4 35.3 6.8 3797	18 78	 8.4 13.5 45.2 35.7 5.9 3950	16 81	 8.0 13.0 44.2 36.4 7.0 4048	22 72	 7.1 11.2 47.2 33.5 3.8 2264	31 65	
Clinical Chemistry	Not performed								
Urinalysis	Not performed								
Organ weights -kidneys (% bw) -liver (% bw) -adrenals (% bw)	 0.012	1.38 5.40 0.025	 0.012	1.42 5.36 0.026	 0.015	1.39 5.44 0.025	 0.016	1.81 6.10 0.034	
Pathology									
- *macroscopy* -dermatitis -spleen enlargement -lymph node enlargement	 16 9 9	 2 4 4	 11 9 8	 2 6 3	 15 13 13	 4 7 5	 35 24 27	 36 24 23	

- *microscopy*									
-degeneration sciatic nerve (early death)							2		
-dermatitis	22/66	5/75					15/16	12/20	
-spleen, extramedullary haematopoiesis	17/66 9/66	24/75 11/75					13/16 9/16	9/20 7/20	
-lymph node, lymphoid hyperplasia	17/66	21/75					13/16	17/20	
-bone marrow, myeloid hyperplasia									

* $p < 0.0001$

Four high dose males were found dead during treatment weeks 3-8 (12.5 mg/kg bw/day). Three additional high dose males were found dead during treatment weeks 9-11 (7.5 mg/kg bw/day). Prior to death some mice showed tremors and vocalization (see below). Treatment-related skin lesions were seen in two of these mice, and consisted of slight degeneration of the sciatic nerve (vacuolation). The incidence of mortality from weeks 12 to 51 in high dose animals was similar to controls. After this time, the incidence of mortality increased in high dose animals, partly due to systemic infection occurring as a consequence of skin lesions (see below). Total mortality in the high dose group was 68% and 60% for males and females, respectively, compared to 34% and 25% for control males and females, respectively.

In high dose males, tremors and vocalization were observed between weeks 5 to 16. In high dose females vocalization was observed between weeks 16 to 34. From week 14 on, a fine fasciculating tremor of the forequarter/forelimbs (and rarely the hindquarter/hindlimbs) was observed in the high-dose group when the mice were suspended by their tails during handling. Occasionally sharp myoclonic movements were observed as long as the animals were held by the tail. After week 18, the incidence of these signs increased gradually and affected a large number of mice in the high-dose group. The observed skin lesions were characterized by cracking, exudate formation, hair loss and scabbing. Cervical region, ears and eyes were mostly affected. These lesions showed heavy growth of *Staphylococcus intermedies* and/or *Streptococcus Group G*. These lesions, observed most prominent in high dose mice, were observed from week 5 on and became more prominent after 6 months. This skin infection problem, which was exacerbated in high dose animals, was attributed to the poor clinical condition (e.g. markedly lower weight gain) and stress in high dose animals owing to toxicity. Therefore, it was considered to be a secondary effect of toxicity and not a direct test substance related effect. The higher incidence of infectious processess is also considered to be responsible for the proportionately higher incidence of histopathological changes in the spleen, lymph nodes and bone marrow in the high dose group (see below).

Decreased weight gain/body weight loss was observed in high dose mice during the first year. Compared to controls, the average weight gains from one year until termination of the study were approximately 50-60% and 60-70% less in high dose males and females, respectively (statistically significant).

In blood of high dose mice changed numbers of segmented neutrophils, leucocytes and lymphocytes were observed.

Relative organ weights of kidneys (f), liver (f) and adrenals (m, f) were increased in these high dose mice (probably related to lower bodyweights).

In two high dose male mice given 12.5 mg/kg bw/day degeneration of the sciatic nerve (characterized by vacuolation and the presence of small myelin balls in the nerve fibre) was seen. Infectious processes (dermatitis, abscesses and suppurative lesions in viscera) were most prevalent in mice that did not survive to termination.

In high dose mice, gross pathology showed increased incidences in dermatitis, spleen enlargement and lymph node enlargement. These effects were confirmed by microscopic examination (dermatitis, spleen extramedullary haematopoiesis, lymph node lymphoid hyperplasia). These changes in high dose animals were observed at terminal necropsy as well as in early deaths.

There was no treatment-related increased incidence in tumours observed.

Table 3.9.1.2-1 Tumor incidences from a 79 week oral toxicity study in mice, female mice

		Control (0)	**Control (0)**	**0.25 mg/kg bw/day**	**1.00 mg/kg bw/day**	**5.00/ 2.50 mg/ kg bw/day**		
No. of animals tested		**50**	**50**	**50**	**50**	**50**	**Trend p-value (before adjustment)**	**Trend adjusted for multiple tumor sites**
1	Uterus – polyp	0	1	0	1	1	0.353	NS
2	Uterus - leimyoma	2	1	2	0	1	0.491	NS
3	Stomach – glandular mucosa, adenoma	1	0	0	1	0	>0.500	NS
4	Lung - adenoma	4	5	6	3	0	0.005N	SIGa (p = 0.021N)
5	Lung - adenocarcinoma	1	1	3	2	0	0.094N	NS
6	lymphoma	1	1	2	2	0	0.064N	NS
7	Eye – harderian gland, adenoma	3	1	1	5	0	0.152N	NS
8	Adrenal – benign spindle cell tumor	0	2	0	0	0	0.191N	NS

a significant level assessed based on the number of tumor sites, k1-4, for which a more extreme positive p-value should have been observed

SIG statistically significant, P > 0.05

NS not statistically significant, P> 0.05

N indicates a reverse carcinogenic effect

Table 3.9.1.2-2 Tumor incidences from a 79 week oral toxicity study in mice, male mice

		Control (0)	**Control (0)**	**0.25 mg/kg bw/day**	**1.00 mg/kg bw/day**	**5.00/ 2.50 mg/ kg bw/day**		
No. of animals tested		**50**	**50**	**50**	**50**	**50**	**Trend p-value (before adjustment)**	**Trend adjusted for multiple tumor sites**
1	Testis – benign interstitial cell tumor	0	0	2	0	0	>0.500	NS
2	Liver - adenoma	9	6	5	4	1	0.025N	NSa (p = 0.118N)
3	Liver - carcinoma	3	3	7	2	2	0.312N	NS
4	Lung - adenoma	7	12	4	5	3	0.055N	NS
5	Lung - adenocarcinoma	5	2	1	1	1	0.085N	NS
6	lymphoma	1	1	1	0	0	0.083N	NS
7	Eye – harderian gland, adenoma	2	3	2	1	0	0.086N	NS
8	Adrenal – benign pheochromocytoma	1	0	2	0	0	0.233N	NS
9	Stomach – nonglandular mucosa, papilloma	0	1	1	0	0	0.417N	NS
10	Thyroid – follicular	0	1	0	1	0	0.486N	NS

	adenoma							
11	Skin - fibroma	1	0	1	1	0	>0.500N	NS

a significant level assessed based on the number of tumor sites, k1-4, for which a more extreme positive p-value should have been observed

SIG statistically significant, P > 0.05

NS not statistically significant, P> 0.05

N indicates a reverse carcinogenic effect

Acceptability

Exposure scenarios of high dose males and females are different: high dose males exposure exists of three periods with decreasing doses, ending with 5.0 mg/kg bw/day; high dose female exposure exists of two periods, ending with 7.5 mg/kg bw/day.

Room temperature, humidity and air changes were not reported. Several deviations from GLP were reported in the GLP statement.

In spite of abovementioned deviations from OECD 451, the study is considered acceptable as carcinogenicity study.

Conclusions

Based on increased mortality, marked decreased weight gain, clinical signs of neurotoxicity, increased incidence of skin lesions, changes in haematological parameters and increased relative organ weights observed in high dose mice, the NOAEL for general toxicity in this study is 2.5 mg/kg bw/day. No treatment-related increase in tumour incidence was observed in mice.

3.9.2 Human data

No data available.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

For an overview of mutagenicity studies please refer to section 3.8.

3.9.4 Other data (e.g. studies on mechanism of action)

No data available.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1 - Oral range finding

Study reference: B.6.6.1, STUDY 1

Characteristics

Type of study	:	Oral range finding		:	Orally, by gavage or in the diet from GD 0 to LD 21
Year of execution	:	1992	Doses	:	Gavage: 0, 0.1, 0.7 and 5.0 mg/kg bw/day [a] Diet, 0, 1, 7 and 50 ppm, approximately equal to 0, 0.1, 0.7 and 5.0 mg/kg bw/day [a]
Test substance	:	MK-0243: L-656,748-038W002 Purity: 94.2% by HPLC	Vehicle	:	For gavage: Deionized water
Route	:	Oral, gavage and diet	GLP statement	:	Yes
Species	:	Rat, Sprague-Dawley Crl:CD(SD) Br strain	Guideline	:	None
Group size	:	12 mated females/dose	Acceptability	:	Acceptable as range-finding study
			$NOAEL_{SYST}$	:	Range finding study
			$NOAEL_{REPR}$	:	

[a] Expressed as base (factor 1.14)

Study design

Groups of 12 mated female rats received the test substance, either by gavage or in the diet from gestational day (GD) 0 to lactational day (LD) 21. Control groups received either vehicle (gavage) or unmedicated chow. The gavage doses of MK-0243 were 0, 0.1, 0.7 or 5.0 mg/kg bw/day. The dietary doses were 0, 1, 7 or 50 ppm, and were given in such amounts as to reach intended dose levels of 0, 0.1, 0.7 or 5.0 mg/kg bw/day. Body weights were recorded at several time points during gestation and lactation. Food consumption over 4-day periods was measured from GD 0 to LD 12. Animals were checked daily for clinical signs. The gavage-treated females were checked within 1-5 h after dosing. At the day of birth, all pups were counted, weighed, sexed and examined for gross abnormalities. Between LD 21-26 females that delivered were killed and the uterus was examined. High dose gavage-treated females were killed between LD 8-15 due to excessive pup mortality. The brain, spinal cord and sciatic nerves of all females dosed by gavage were examined macroscopically. In the control and high-dose gavage-treated females the brains were weighed and brains, spinal cords and sciatic nerves were histologically examined. The diet-treated females were not further examined.

All pups were examined daily for mortality and morbidity. Pup weight was recorded on LD 4, 7, 14 and 21. On LD 4 litters were culled to 4 male and 4 female pups. At LD 21 the pups were killed, brains were weighed and the brain, spinal cord and sciatic nerves were examined macroscopically. These tissues of the control, high-dose pups (diet-treated) and mid-dose pups (gavage and diet-treated) were examined histologically.

Results

Table 3.10.1.1-1 Results from gavage treatment with emamectine (MK-0243) in a range-finding study for reproductive toxicity

	Dose (mg/kg bw/day)	0		0.1		0.7		5.0		dr
	Sex	m	f	m	f	m	f	m	f	
F0 animals	Mortality	no treatment-related effects								
	Clinical signs	no treatment-related effects								
	Body weight gain GD 0-4 GD 8-12 LD 0-4 LD 4-8						+33%[is] -32%[ds]		+42%[is] -61%	

									ds -78% ds	
	Food consumption GD 0-4 GD 8-12 LD 0-4 LD 4-8 LD 8-12						+13%[i] s		+17% is +11% is -20% ds -38% ds -42% ds	
	Gestation	no treatment-related effects								
	Brain weights [A]	no treatment-related effects								
	Pathology [A]									
	- macroscopy	no treatment-related effects								
	- microscopy	no treatment-related effects								
pups	Litter size	no treatment-related effects								
	Mortality LD 1-4 LD 5-7 LD8-14							9% [is] 21% [is] 42% [is]		
	Clinical signs							is[B]		
	Body weight (gain) - LD 0 - LD 14							-5% [ds] -62% [ds]		
	Brain weight	no treatment-related effects						N.D.	N.D.	
	Pathology									
	- macroscopy	no treatment-related effects						N.D.	N.D.	
	- microscopy (weanlings)			N.D.		no treatment-related effects		N.D.	N.D.	

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly
N.D. = not determined
[A] High-dose females were killed between LD 8 and 15 due to high pup mortality.

[B] Tremors were observed at LD11-12 in about one-third of the pups. In addition, a few pups were pale, cold, weak and/or breathing shallow.

Table 3.10.1.1-2 Results from dietary treatment with emamectine (MK-0243) in a range-finding study for reproductive toxicity

	Dose (ppm)	0		1		7		50		dr
	Sex	m	f	m	f	m	f	M	f	
F0 animals	Mortality	no treatment-related effects								
	Clinical signs	no treatment-related effects								
	Body weight gain GD 0-4 GD 8-12 LD 4-8								+68%[i] s -21% ds -67% ds	
	Food consumption GD 4-8 LD 8-12								+13% is -26% ds	

	Gestation	no treatment-related effects								
pups	Litter size	no treatment-related effects [A]								
	Mortality	no treatment-related effects								
	Clinical signs							is [B]		
	Body weight gain - LD 14 - LD 21							-34% ds -52% ds		
	Brain weight							-18% ds	-16% ds	
	Pathology									
	Macroscopy	no treatment-related effects								
	microscopy (weanlings) - CNS - PNS			N.D. N.D.		no treatment-related effects no treatment-related effects		12/16 is C no treatment-related effects		

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly
N.D. = not determined

[A] A reduction in post implantation survival was mainly due to 1 female with 100% post implantation loss. This was considered not treatment-related, since post implantation survival in the other high-dose females was comparable to controls.

[B] At LD11-12 tremors were observed in all pups of the high-dose group. In addition a few pups in the high dose groups were pale, cold, weak and/or breathing shallow.

[C] In 12 out of 16 examined pups neurons within the pons, and spinal gray matter were swollen. The swollen neurons had an increased amount of eosinophilic cytoplasm, central chromatolysis and the nucleus was displayed to the periphery of the cell body.

Additional information: The lower fecundity (and fertility) values across all treated groups in the first mating was not reproduced in the following two matings, suggesting that it was not treatment related. In addition there was no dose response. The reduced fecundity and fertility at the high dose level is considered to be treatment related, and to be a secondary consequence of neurotoxicity to the male leading to ineffective copulation.

The reduced number of pups per litter at the high dose level is unlikely to be treatment related, since there was no clear dose response, and the effect was not reproduced across the next two matings, suggesting that this apparent reduction at the high dose in the F1a generation probably reflects normal variation in this parameter.

	Dietary Concentration of emamectin (mg/kg/day)			
Observation	**0**	**0.1**	**0.6**	**3.6/1.8**
F1A Litter				
Live pups per litter	14.1	15.0	14.7	13.0*
F1B Litter				
Live pups per litter	14.9	14.5	16.1	15.1
F2 Litter				
Live pups per litter	13.9	13.6	15.4	14.7

Acceptability

Acceptable as range-finding study.

Conclusions

Treatment-related maternal toxicity (body weight loss, decreased food consumption) was observed in the high-dose gavage- and diet-treated groups. In the offspring of the high-dose gavage- and diet-treated groups toxicity was evidenced by clinical signs (tremors) and reduced body weight gains, as well as (gavage only) increased mortality between LD8-15. In pups of the high-dose diet-treated group a reduced brain weight and neuronal degeneration in the brain and spinal cord were additionally observed.

3.10.1.2 STUDY 2 - Two-generation dietary reproduction study

Study reference: B.6.6.1, STUDY 2

Characteristics

Type of study	:	Two-generation dietary reproduction study	Exposure	:	Dietary
Year of execution	:	1993	Doses	:	0, 0.1, 0.6 and 3.6/1.8 mg/kg bw/day. [a,b]
Test substance	:	MK-0244: L-656,748-052S002 benzoate hydrate salt Purity: >96% by HPLC	Vehicle	:	None
Route	:	Oral, diet	GLP statement	:	Yes
Species	:	Rat, Sprague-Dawley Crl:CD(SD) Br strain	Guideline	:	Resembles OECD 416
Group size	:	33/sex/dose	Acceptability	:	acceptable
			$NOAEL_{PARENTAL}$	:	0.6 mg/kg bw per day
			$NOAEL_{FERT.NO}$	:	0.6 mg/kg bw per day
			$AEL_{DEV.}$	:	0.6 mg/kg bw per day

[a] Expressed as base (factor 1.15)

[b] The high-dose level was lowered to 1.8 mg/kg bw/day for F0 and F1A females on GD0.

Study design

Rats (33/sex/dose) received the test substance in the diet from about 9 weeks premating. The dietary doses were adjusted 1-2x/week to achieve the desired doses of 0, 0.1, 0.6 and 3.6 mg/kg bw/day. The high-dose level was lowered to 1.8 mg/kg bw/day on GD0 for F0 females producing the F1b generation, and on GD0 for F1a females producing the F2 generation. Premating body weights were recorded weekly and food consumption was recorded over 6-day periods. Subsequently, in females body weights were recorded at GD 0, 4, 8, 12, 16, 20 and 24 and on LD 0, 4, 8, 12, 16, 20 and 21. During gestation and lactation food consumption was measured over 4-day periods. The animals were examined daily for clinical signs. At birth pups were counted, sexed, examined and weighed. At LD 4 litters were culled to 4 pups/sex, the other pups were discarded. Pup weight was measured on LD 4, 7, 14 and 21. On LD 21, 1 pup/sex from each litter from the first 25 litters of the F1a generation was randomly selected to produce the F2 generation. The F0 generation was mated for a second time to produce an F1b generation. After weaning of the F1b pups, i.e.

after 25-27 weeks of treatment, the F0 females were killed, necropsied and the uterus was examined. Reproductive tissues and gross lesions from control and high dose animals and brain, spinal cord and sciatic nerves of all parental animals were examined histologically. All pups not selected for the production of the F2 generation were discarded without further examination. All F0 males were killed after 24 weeks of treatment and reproductive and nervous tissues were examined histologically.

After weaning, F1a animals selected to produce the F2 generation were examined daily for mortality and clinical signs. The high dose level was maintained at 3.6 mg/kg bw/day during the post weaning period up to GD0, when it was lowered to 1.8 mg/kg bw/day. Measurements of the parameters were the same as for the F0 parental animals.

Results

Table 10.1.2-1 Results from dietary treatment with emamectin (MK-0244) in a 2-generation study for reproductive toxicity

	Dose (ppm)	**0**		**0.1**		**0.6**		**3.6/1.8**		**dr**
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals	Mortality	no treatment-related effects								
	Clinical signs	no treatment-related effects								
	Body weight gain - Premating - During/after 2nd mating							 -21% ds	+16% is	
	Food consumption - Premating - during 1st lactation - during 2nd lactation								+ 13% is - 28% ds A - 22% ds A	
	Mating, fertility, gestation - 1st mating fecundity B -2nd mating fecundity		 91% 88%		 71% d 88%		 76% d 84%		 71% d C 74% d C	
	Oestrus cycle	N.D.								
	Sperm parameters	N.D.								
	Organ weights	N.D.								
	Pathology									
	- macroscopy	no treatment-related effects								
	- microscopy D brain spinal cord sciatic nerve							 29/33 is 31/33 is 4/33 is	 23/33 is 5/33 is	
F1a pups	Litter size	no treatment-related effects E								
	Survival index	no treatment-related effects								
	Sex ratio	no treatment-related effects								
	Clinical signs							is F		
	Body weight - at birth	no treatment-related effects								
	Body weight gain - at LD 14 - at LD 21							 -9% ds -29% ds	 -10% ds -29% ds.	
	Organ weight	N.D.								

	Dose (ppm)	**0**		**0.1**		**0.6**		**3.6/1.8**		**dr**
	Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
	Pathology									
	- macroscopy	no treatment-related effects								
	- microscopy (weanlings)	N.D.								
F1b pups	Litter size	no treatment-related effects								
	Survival index	no treatment-related effects								
	Sex ratio	no treatment-related effects								
	Clinical signs							is[F]		
	Body weight (gain)	no treatment-related effects								
	Organ weight	N.D.								
	Pathology									
	- macroscopy	no treatment-related effects								
	- microscopy (weanlings)	N.D.								
F1 animals	Mortality	no treatment-related effects								
	Clinical signs							is[G]		
	Body weight	no treatment-related effects								
	Food consumption	no treatment-related effects								
	Mating, fertility, gestation Fecundity		80%		87%		95%		52% ds[C]	
	Oestrus cycle	N.D.								
	Sperm parameters	N.D.								
	Organ weights	N.D.								
	Pathology									
	- macroscopy	no treatment-related effects								
	- microscopy [H] brain spinal cord							23/25 is 23/25 is	18/27 is 7/27 is	
F2 pups	Litter size	no treatment-related effects								
	Survival index	no treatment-related effects								
	Sex ratio	no treatment-related effects								
	Clinical signs							is[I]		
	Body weight gain							ds[J]		
	Pathology									
	- macroscopy	no treatment-related effects								
	- microscopy	N.D.								

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly
N.D. = Not determined

A Maximal reduction as compared to controls during a 4-day interval in this phase of the test

B Fecundity index: pregnant females/mated females.

C A slight decrease in fecundity index (pregnant females/mated females) was observed in all treatment groups during the first mating. These values were within the historical control range. Animals that had not produced a pregnancy in the first mating were paired with known fertile animals during the second mating. No effect on fecundity was observed in the low-and mid-dose groups during the production of the F1b and F2 generation. Therefore the reduced fecundity observed in the low and mid-dose animals in the first mating was considered not related to treatment. In the second mating again a decreased fecundity was observed in the high-dose animals. Analysis of the affected sex indicated that approximately 20% of the high-dose females failed to produce a pregnancy after mating occurred. Since a reduced fecundity was also observed in the high-dose group producing the F2 generation, it is considered that the observed reduced fecundity at the high dose is treatment-related.

D In the brains and spinal cords of animals of the high dose group very slight to slight degeneration of neurons was observed. In the males of the high dose group a few animals had very slight degeneration of the sciatic nerve.

E A slight but statistically significant reduction in number of pups per litter was observed in the F1a group (high dose 13.0 pups/litter, control 14.1 pups/litter). However, since this was within the historical control range and since in the F1b and F2 pups actually a slight increase in litter size was observed at the high dose this finding is considered incidental and not treatment-related.

F In all F1a litters of the high dose group a number of pups displayed head or whole body tremors, hindlimb extensions, and limited use of hind limbs, usually starting between LD 7-12.

In the high dose F1b litters, where the dose was lowered from 3.6 to 1.8 mg/kg bw/day from GD 0 to LD 21, tremors and limited use of hindlimbs was only observed in 5 and 7 litters, respectively.

G After weaning, all high dose pups selected to produce the F2 generation displayed hindlimb splays and about half of the pups displayed whole body tremors. The hindlimb effects and tremors were not observed after week 5 and 4 after birth, respectively.

H In the brains and spinal cords of animals of the high dose group very slight to slight degeneration of neurons was observed.

I In the high dose group, where the dose was lowered from 3.6 to 1.8 mg/kg bw/day from GD 0 to LD 21, tremors and limited use of hindlimbs was observed in 1 litter only.

J Slight (5-9%) but statistically significant reductions in body weight were observed in the high dose pups (both sexes) at LD14 and LD 21, respectively. At LD 4 a statistically significant reduction (10%) in male pup weight was observed in the high dose group.

Acceptability

The study is considered acceptable

Conclusions

The NOAEL for parental toxicity was 0.6 mg/kg bw/day, on the basis of a reduced body weight gain in F0 males during/after 2nd mating, an increased body weight gain and food consumption in females during premating, a reduced food consumption in females during lactation, and neuronal degeneration in the brain, spinal cord and (3.6 mg/kg bw/day males only) sciatic nerve, observed at 3.6/1.8 mg/kg bw/day.

The NOAEL for developmental toxicity was 0.6 mg/kg bw/day on the basis of reduced body weight gain during the lactation period.

The NOAEL for reproductive toxicity was 0.6 mg/kg bw/day on the basis of a reduced fecundity.

3.10.1.3 STUDY 3 - Developmental toxicity

Study reference: B.6.6.2, STUDY 1

Characteristics

Type of study	:	Developmental toxicity	Exposure	:	orally by gavage; days 6-19 of gestation
Year of execution	:	1989-1992	Doses	:	0, 2, 4, and 8 mg/kg bw per day*
Test substance	:	MK-0243: L-656,748-038W002 benzoate salt Purity: 94.2%	Vehicle	:	Deionized water
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) Br strain	Guideline	:	-
Group size	:	25 mated females	Acceptability	:	acceptable
			$NOAEL_{mat}$	:	2 mg/kg bw per day
			$NOAEL_{dev}$	:	2 mg/kg bw per day

* a factor 1.14 was used to calculated the dosages as base compound

Study design

Groups of 25 mated young adult female rats were treated orally, by gavage, with L-656,748-038W (the benzoate salt) in water at dose levels of 0, 2, 4 and 8 mg/kg per day from days 6 through 19 of gestation (day 0 = observation of copulatory plug). Clinical signs were recorded three times daily during the administration period (predose and 1 and 5 h after dosing), and body weights and food consumption were recorded before the start of administration and at 2 day intervals thereafter. Dams were killed on day 20 of gestation and their pregnancy status established. Dams were examined macroscopically, and the number of corpora lutea, the presence of resorptions and dead foetuses were examined. Foetuses were sexed, weighed, and examined for external alterations. Approximately one half of the foetuses from each litter and all externally malformed foetuses were given a visceral examination, and their heads were removed and processed for evaluation of soft tissue alterations. All foetuses were processed and examined for skeletal malformations and variations.

Results

The results of the teratogenicity study are summarized in table 3.10.1.3-1.

Additional information on maternal body weights and food consumption, and ossification data of fetuses are presented in tables 3.10.1.3-1a to d and Figures 3.10.1.3-1a and b.

Table 3.10.1.3-1 Results from a developmental toxicity study in rats.

	Dose (mg/kg bw per day)	**0**	**2**	**4**	**8**	**dr**
Maternal effects	Mortality	none				
	Clinical signs: - *alopecia* - *ocular discharge* - *mass* [a] - *tremors* - *unkempt coat* - *no faeces* - *convulsions*	 1	 1	 2	 1 1 15 3 2 2	
	Pregnant animals	21	19	21	21	
	Abortions	none				
	Gravid uterine weight	not measured				
	Corpora lutea	Mean data not shown				
	Body weight changes [b]		i days 6-14	i days 6-14 dc days 14-20	dc days 6-20	dr
	Food consumption		i days 8-11	i days 8-11	i days 8-11 dc days 17-20	
	Water consumption	not measured				
	Pathology [c]	No treatment-related findings				
Litter response	Live foetuses	344	315	312	332	
	Foetal weight: m/f	3.84/3.70	3.73/3.58	3.76/3.56	3.69/3.48	
	% Pre implantation loss	7.4	4.6	8.5	10.2	
	No. of resorptions	4	4	6	16	

	Dose (mg/kg bw per day)	**0**	**2**	**4**	**8**	**dr**
	Post implantation loss (% resorp+dead foet)/impl (litter mean))	1.1	1.7	1.9*	4.3	
	Sex ratio (m:f)	1 : 0.96	1 : 0.96	1 : 0.95	1 : 0.94	
Foetus examination	No. of live foetuses	344	315	312	332	
	No. of dead foetuses	0	1	0	0	
	External examinations (no of animals with:) - *micrognathia* - *cleft palate* - *tail malformation* - *hematoma*	 1 2	 1 	 1 	 1 	
	visceral examinations (no of animals with:) - *ventricular hypoplasia* - *agnesis of testis* - *reduced ductus arteriosus* - *diffuse haemorrhagic kidney* - *variation in liver lobation*	 1 	 1 1 1	 1 	 1 	
	Skeletal examinations No. of animals with malformations:	9	2	0	2	
	No. of malformations: - *Thoracic vertebra malf.* - *lumbar vertebra malf.* - *sacral vertebra malf.* - *missing vertebra* - *hypoplastic rib*	 3 2 1 7	 1 1	 	 1 1 1 	
	No. of foetuses with variations:	37	37	44	77	dr
	No. of variations: - *vertebral count* - *wavy rib* - *cervical rib* - *supernumary rib* - *sternebral variation*	 2 1 36	 2 1 36	 5 1 41 1	 4 5 73	 dr
	No. of foetuses with incomplete ossification:	19 (5.5%)	18 (5.7%)	27 (8.7%)	46 (13.9%)	dr
	No. of sites of incomplete ossification: [d]	20	21	32	78	dr

i= increased; dc= decreased stat. significant; dr= dose releated

* this value is calculated by present reviewer, based on total values and not based on litter mean. In the table of the study report the value given was 7.6. This value is considered an erroneous value since there were only 6 resorptions on a total of 318 implants.

a One female had a mass in left thorax, another female in right abdomen.

b In the mid dose group, the overall weight gain (days 0 through 20) was significantly decreased by 5.2%, and during days 14-20 by 13%. In the high dose group statistically significant decreases in body weight were seen during the whole gestational period (days 4-14; 14-20 and 6-20). The overall reduction in body weight gain was 33.3% and during days 14-20 35%.

c The masses found in the mid dose group (see under legends note a) were found to be a galactocoele in the left thorac region, and a mammary adenoma in the right abdominal area.

d The main sites of incomplete ossification were: cervical vertebra, skull bone, and pelvic bone.

Table 3.10.1.3-1-1a Average maternal bodyweights (g)

	CONTROL	2 MG/KG/DAY	4 MG/KG/DAY	8 MG/KG/DAY
GESTATIONAL PERIOD				
DAY 0	263 (21)	267 (19)	275 (21)	265 (21)
DAY 6	301	306	312	300
DAY 8	308	318	326	312
DAY 10	318	329	335	322
DAY 12	331	343	350	327
DAY 14	344	357	360	330
DAY 16	365	377	377	340
DAY 18	397	409	406	364
DAY 20	436	448	439	390

(N) = GROUP SIZE AND APPEARS ONLY IF DIFFERENT FROM PREVIOUS N. SEE INDIVIDUAL TABLE FOR EXCLUSIONS.

Table 3.10.1.3-1b Maternal bodyweight gain of pregnant females (g)

	Dose Level of MK-0243 (mg/kg/day)			
Gestation Period	**0 (control)**	**2**	**4**	**8**
Days 6 to 14	42	51	48	30^{S}
Days 14 to 20	92	90	80^{S}	60^{S}
Days 6 to 20	135	141	128^{Q}	$90^{L, Q, A}$

S = trend statistically significant ($P \leq 0.05$) through indicated dose.
L, Q, A = results of trend analyses for linear, quadratic and average time responses are indicated by the corresponding letter (L, Q and A respectively) if statistically significant ($P \leq 0.05$) through indicated dose. Trend analyses for linear and average time responses were performed with an adjustment for day 6 weights.

Figure 3.10.1.3-1a Average maternal body weights

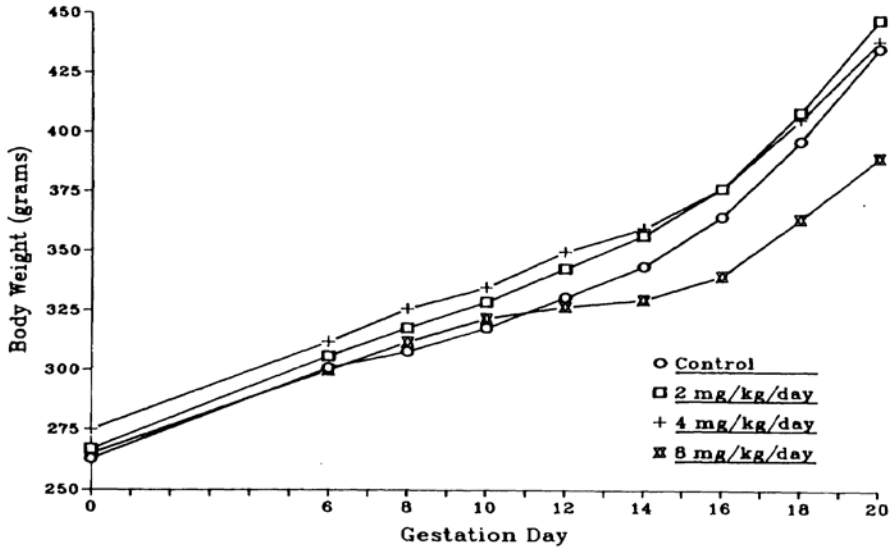


Table 3.10.1.3-1c Maternal food consumption, group means (g/day)

	Dose Level of MK-0243 (mg/kg/day)			
Gestation days	**0 (control)**	**2**	**4**	**8**
5	26	25	26	25
8	26	28	30[S]	29[S]
11	27	29[S]	31[S]	32[S]
14	28	30	30	28
17	30	31	32	26[S]
20	29	30	29	24[S]

S = trend statistically significant ($P \leq 0.05$) through indicated dose.

Figure 3.10.1.3-1b Average maternal food consumption

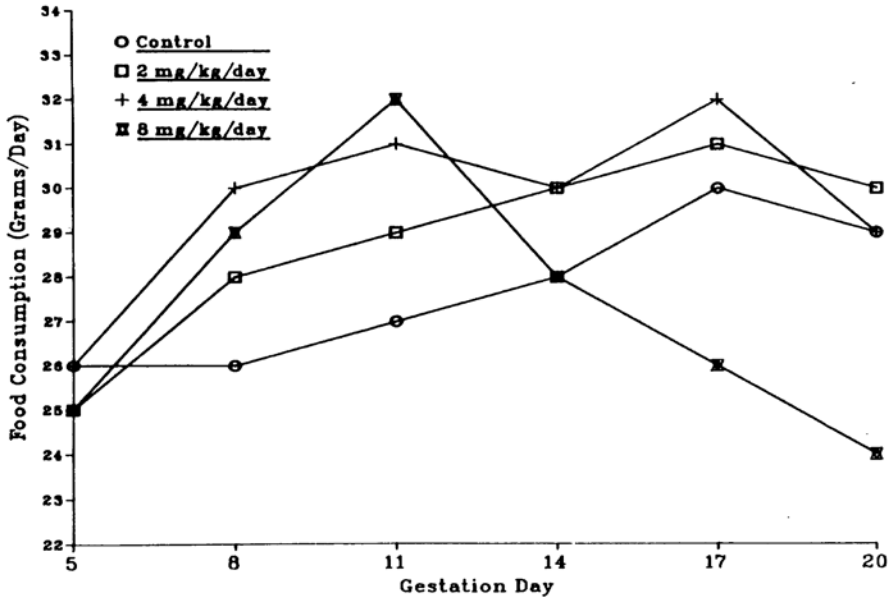


Table 3.10.1.3-1d Summary of fetal ossification data

SUMMARY OF FETAL OSSIFICATION DATA

TREATMENT GROUP:	CONTROL	2 MG/KG/DAY	4 MG/KG/DAY	8 MG/KG/DAY
FETUSES				
NUMBER EXAMINED	344	315	312	332
NUMBER WITH SITES OF INCOMPLETE OSSIFICATION	19	18	27	46
NUMBER OF SITES OF INCOMPLETE OSSIFICATION	20	21	32	78
NUMBER OSSIFIED SACROCAUDAL VERTEBRAE (LITTER MEAN)	8.0	7.9	8.0	8.0
LITTERS				
NUMBER EXAMINED	21	19	20	21
NUMBER WITH SITES OF INCOMPLETE OSSIFICATION	10	10	10	12
SITES OF INCOMPLETE OSSIFICATION				
INCOMP. OSS. CERVICAL VERTEBRA	3	6	7	16^{NS}
INCOMP. OSS. THORACIC VERTEBRA	0	2	2	1
INCOMP. OSS. LUMBAR VERTEBRA	1	1	1	4
INCOMP. OSS. SKULL BONE	0	1	3^{NS}	4^{S}
INCOMP. OSS. RIB	0	0	0	3
INCOMP. OSS. STERNEBRA	14	7	16	15
INCOMP. OSS. PELVIC BONE	2	4	3^{NS}	35^{S}

S = TREND STATISTICALLY SIGNIFICANT ($P \leq 0.05$) THROUGH INDICATED DOSE.
NS = TREND NOT STATISTICALLY SIGNIFICANT ($P > 0.05$) THROUGH INDICATED DOSE.

No mortality and abortions occurred in the study. Treament-related clinical signs in dams were observed in the high dose only. Tremors were first observed between days 10 through 18 and persisted in all animals involved until termination. Convulsions, few or no faeces and unkempt coats were observed in 2 high dose dams about 6-7 days after the initiation of tremors. Unkempt coat was also observed in one other dam having tremors.

From days 6 - 14 of gestation, minor test substance-related increases in weight gain occurred at 2 and 4 mg/kg per day and were associated with increases in average food consumption in the same period. The study authors considered these effects as treatment-related since the effects on body weight (food consumption not measured) had also occurred (in the same period) in an earlier performed range-finding study at dose levels of 2.5 and 5 mg/kg bw per day (report not available to present reviewer). These effects on body weight and food consumption were considered of minor toxicological significance. However, treament-related reductions in overall body weight gain over the gestation period were observed in the mid dose (5.2%) and high dose (33.3%) group and a statistically significant decrease in food consumption was observed in the high dose dams during the last days of treatment (days 17-20).

The number of resorptions in the high dose group was increased compared to the controls. This was partly due to 2 litters with 4 and 6 resorptions respectively. The increase was considered to be normal biological variation according to the study authors, since the percentage resorptions plus dead foetuses per implants (4.3% for the high dose group) were close to the mean value (4.0%) of historical control groups of recently performed studies. These historic control values were not available to the present reviewer, nonetheless, the RMS considers the increase of the number of resorptions in the high dose group as treatment-related.

Mean live foetal weight was slightly decreased in the high dose group (m/f). This decrease was not statisitically significant but was considered treatment-related by the study authors.

Only some incidental findings, but no treatment-related findings were observed during external foetal examinations and visceral examinations. There were no treatment-related skeletal malformations. However, the number of foetuses with skeletal variations was increased in the high dose group. This was mainly due to increases in the number of wavy ribs, and supernumerary ribs. An increase in the number of foetuses and in the number of sites with incomplete ossification was observed in the mid and high dose group. This effect was in some cases significant in only the high dose group. The effect is possibly related to decreased fetal weight and not a direct effect of emamectin on skeletal maturation. However, it should be noted that the observed decrease in fetal body weight was only minor and not significantly different from control animals. Moreover, in the mid-group dams a slightly decreased body weight gain was observed from GD 14 onwards.

At the high dose level, a remarkable reduction of body weight was observed in the dams (33%). The fetal effects observed in the high dose group might thus be correlated to the BW effects in the dams.

Acceptability

The study was performed in accordance with OECD guideline 414.
Deviations: uterus weight was not determined.

Conclusions

Maternal toxicity was observed mainly in the high dose group with clinical signs of neurotoxicity and a decrease in body weight gain. A slight decrease in body weight gain was also observed in the mid dose group and therefore, the NOAEL for maternal toxicity is established at 2 mg/kg bw per day.
The NOAEL for embryo/foetotoxicity is also established at 2 mg/kg bw per day, based on dose-related increases in the number of foetuses with incomplete ossification and in the number of sites with incomplete ossification. Additionally, a slight decrease in foetal weight, an increase in the number of resorptions, and an increase in skeletal variations (wavy rib and supernumary ribs) were observed in the high dose group, of which the dams showed excessive toxicity (reduced bw gain of 33%). There was no evidence of a direct and irreversible teratogenic effect in the rat.

Remark:
The study authors established a maternal NOAEL of 1.25 mg/kg bw per day based on slight increases in body weight at 2.5 and 5 mg/kg bw per day in the preliminary teratogenicity study. The NOAEL for developmental toxicity was established at 4 mg/kg bw/day.
The notifier established a maternal NOAEL at 2 mg/kg bw/day and a foetotox NOAEL at 4 mg/kg bw.

3.10.1.4 STUDY 2 - Developmental toxicity –range finding study

Study reference: B.6.6.2, STUDY2a

Characteristics

Type of study	:	Developmental toxicity – range finding study	Exposure	:	orally by gavage; days 6-18 of gestation
Year of execution	:	1989-1992	Doses	:	0, 2, 4, 6 and 8 mg/kg bw per day*
Test substance	:	MK 0243; L656,748-038W002 Benzoate salt Purity 96.2%	Vehicle	:	deionized water
Route	:	Oral	GLP statement	:	no
Species	:	Rabbit NZW	Guideline	:	-
Group size	:	10 pregnant females/dose**	Acceptability	:	acceptable

* a factor 1.14 was used to calculated the dosages as base compound

** one female in the 4 mg/kg group was misdosed on GD 6, removed from the study and replaced by another female.

Study design

Groups of 10 artificially inseminated young adult female rabbits were treated orally, by gavage, with L-656,748-038W (the benzoate salt) in water at dose levels of 0, 2, 4, 6 and 8 mg/kg per day from days 6 through 18 of gestation.

Dose levels were based on results of a previous oral range finding study in non-prognant rabbits. In that study the high dose group (10.96 mg/kg/d) had to be euthanised on drug day 6 to 8 due to adverse physical signs, including tremors in all animals and body weight loss in all animals. There were no treatment-related effects in the groups given 5.48 mg/kg/day and lower.

Clinical signs were recorded twice daily during the administration period (at dosing and 1-5 h after dosing) and daily thereafter. Body weights were recorded on days 0 and 28 and at 2-day intervals during the administration period. Food consumption was measured every third day. On gestation day 19, approximately 24 hours after the last dose, all females (non-fated) were bled for hematology and serum biochemical determinations. Does were killed on day 28 of gestation and their pregnancy status established. The number of corpora lutea, the presence of resorptions and dead foetuses were examined. Foetuses were sexed, weighed, and examined for external alterations. Following euthanasia, two misshapen heads of fetuses in the high dose group were removed and processed for evaluation.

Results

The results of the teratogenicity study are summarized in table 3.10.1.4-1.

Table 3.10.1.4-1 Results from a developmental toxicity study in rabbits.

	Dose (mg/kg bw per day)	**0**	**2**	**4**	**6**	**8**	**dr**
Maternal effects	Mortality	none					
	Clinical signs: - *soft feces* - *small feces* - *no urine* - *tremors* - *mucoid discharge*	 0 0 0 0 0	 2 0 0 0 0	 1 0 0 0 0	 1 0 0 0 0	 5 2 1 1 1	
	Pregnant animals	9	10	8	9	10	
	Resorptions	3	14	2	3	10	
	Implants	71	79	63	55	74	
	Implants/pregnant female	7.9	7.9	7.9	6.1	7.4	
	Body weight changes				dc $^{\text{days 14-19}}$	dc $^{\text{days 14-19}}$	
	Food consumption				dc $^{\text{day 16 and 19}}$	dc $^{\text{day 16, 19 and 22}}$	
	Water consumption	Not measured					
	Hematology	No treatment-related findings					
	Serum biochemistry - *glucose (mg/dl)* - *cholesterol (mg/dl)* - *potassium (meq/l)* - *triglycerides*	 117 25 4.2 207	 128 32 3.7 139	 133 27 3.6 149	 127 31 3.5 138	 131 39 3.7 166	
	Pathology	Not performed					
Litter response	Live foetuses	67	64	61	51	63	
	Foetal weight: m/f	38.0	37.0	37.1	38.0	35.9^{ns}	
	No. of dead foetuses	1	1	0	1	1	
Foetus examination							
	External examinations						
	No. of foetuses with malformations - *cleft palate*	 0	 0	 0	 0	 1	
	No. of foetuses with variations:	0	0	0	0	0	
	Visceral examinations						
	No. of foetuses examined	0	0	0	0	2	
	No. of foetuses with malformations - *hydrocephalus*	 0	 0	 0	 0	 2	
	No. of foetuses with variations:	0	0	0	0	0	
	Skeletal examinations	Not performed					

dc= decreased stat. significant; dr= dose releated; ns= not significant for trend test

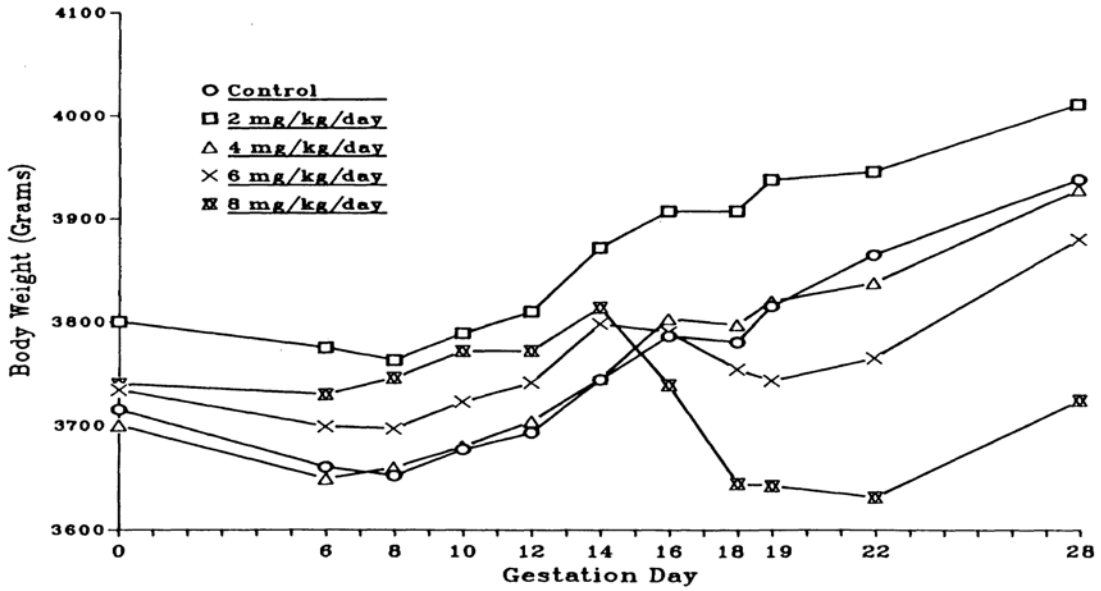


Figure 3.10.1.4-1 Average maternal body weights

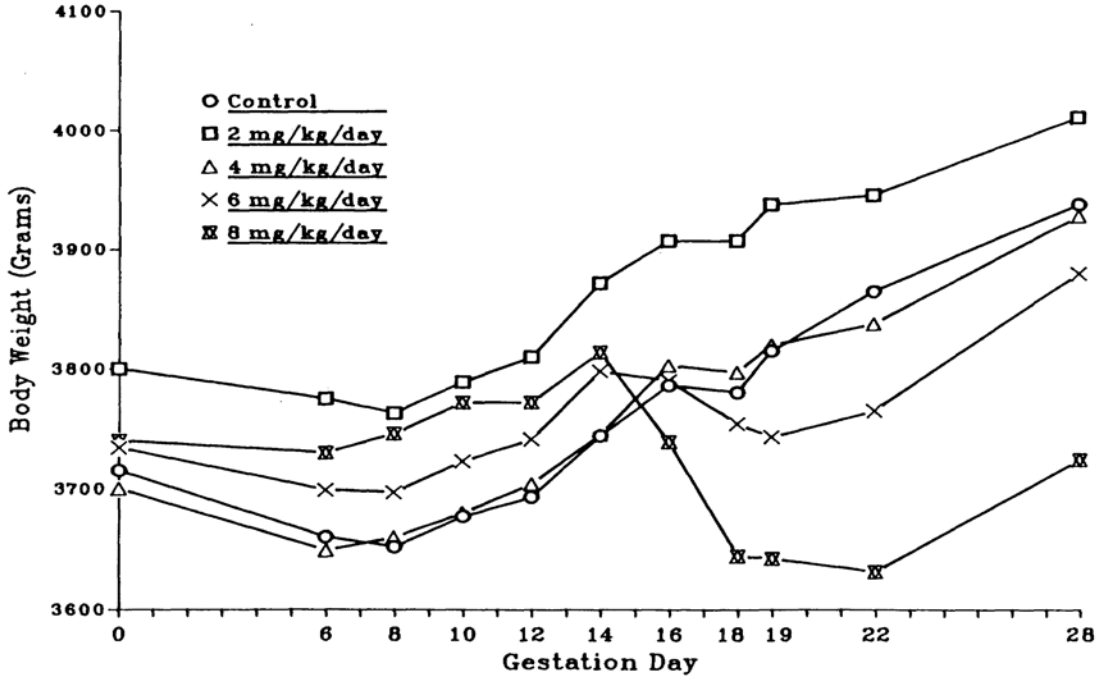


Figure 3.10.1.4-2 Average maternal food consumption

No mortality occurred in the study. Treatment-related clinical signs in does were observed in the high dose group and consisted of tremors (1 female, d19-28), soft and/or small feces (3 females) and no urine on one day in 1 of these females.
There were treatment-related body weight losses between days 14 and 19 of gestation in the 6 and 8 mg/kg/d groups. Body weight gain was normal between day 6-14 and 19-28. Food consumption was decreased in the 6 and 8 mg/kg/d groups on GD 16 and 19 (-11 and -45%), and on GD 22 in the high dose group (-13%). There were no treatment-related effects on haematology, and the effects seen at serum biochemistry were not dose related, and therefore also considered not treatment-related.
Two fetuses from separate litters in the high dose group had malformations: one fetus (from dam 89-0619) had cleft palate and hydrocephaly, the other fetus (from dam 89-0618) had hydrocephaly.These affected fetuses were from dams which showed the greatest body weight losses during the dosing period and/or had tremors.

CONTINUED
TABLE 10. MK-0243: ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS. TT#89-715-1
INDIVIDUAL BODY WEIGHTS OF FEMALES
8 MG/KG/DAY

FEMALE NUMBER	REPRODUCTIVE STATUS	FEMALE BODY WEIGHTS (GRAMS) GESTATION DAY 0	6	8	10	12	14	16	18	19	22	28
890610	PREG	4098	4131	4125	4144	4166	4217	4124	4000	3942	3903	3944
890611	PREG	4034	4012	4022	4077	4053	4130	4137	4117	4145	4136	4293
890612	PREG	3829	3821	3859	3866	3884	3953	3836	3702	3704	3620	3667
890613	PREG	3780	3806	3833	3845	3845	3945	3727	3560	3570	3670	3838
890614	PREG	3703	3694	3694	3745	3752	3828	3727	3688	3700	3659	3623
890615	PREG	3785	3758	3716	3797	3814	3880	3845	3820	3838	3876	4014
890616	PREG	3657	3655	3674	3670	3698	3714	3703	3634	3698	3718	3804
890617	PREG	3658	3642	3678	3692	3694	3760	3750	3727	3725	3719	3851
890618	PREG	3480	3418	3487	3465	3424	3432	3330	3172	3096	3155	3231
890619	PREG	3381	3364	3370	3418	3392	3283	3212	3025	2999	2850	2975

CONTINUED
TABLE 11. MK-0243: ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS. TT#89-715-1
INDIVIDUAL FOOD CONSUMPTION (GRAMS/DAY) OF FEMALES
8 MG/KG/DAY

FEMALE NUMBER	REPRODUCTIVE STATUS	1-DAY INTERVAL ENDING ON GESTATION DAY: 1	4	7	10	13	16	19	22	25	28
890610	PREG	125	127	126	127	123	26	7	124	134	125
890611	PREG	121	125	125	128	124	123	126	124	129	116
890612	PREG	125	124	125	133	123	15	11	120	123	124
890613	PREG	123	124	124	132	123	1	2	125	126	124
890614	PREG	125	125	124	125	125	125	123	125	126	126
890615	PREG	a	128	125	121	124	122	126	124	124	122
890616	PREG	a	127	124	124	125	126	124	125	124	126
890617	PREG	a	126	125	122	127	120	126	125	124	125
890618	PREG	a	126	125	114	124	41	1	100	124	125
890619	PREG	a	126	124	122	88	1	12	1	129	126
* MEAN		124	126	125	125	121	70	66	109	126	124

* INCLUDES ONLY VALUES FROM PREGNANT FEMALES

[a]NO VALUE SINCE WEIGHT OF UNCONSUMED FOOD COULD NOT BE DETERMINED ACCURATELY DUE TO INADVERTENT EXTRA FEEDING.

CONTINUED
TABLE 12. MK-0243: ORAL RANGE-FINDING STUDY IN PREGNANT RABBITS. TT #89-715-1

INDIVIDUAL PHYSICAL SIGNS REPORT

TREATMENT GROUP & ANIMAL NUMBER	PHYSICAL SIGN	DAY(S) OBSERVED
8.0 MG/KG/DAY		
89-0617	N	0-17, 19-24, 26-28
	Increased Urine	18, pre
	Sediment in Pan	25
89-0618	N	0-16, 19, 21-23, 25-26
	Blood in Pan	17, 18, pre
	Vaginal Discharge, Blood	17, pre
	Soft Feces	20
	Pulling Fur	24, 28
89-0619	N	0-18
	Tremors	19-28
	Small and Black Feces	20-22
	Mucoid Discharge	22
	Soft Feces	23
	Blood in Pan	22, 23

N = Normal
pre = predose
post = postdose

Acceptability
The study was performed as a dose range finding study.

Conclusions
In this dose range finding study, treatment related effects in females were observed at 6 and 8 mg/kg bw/d. In 2 fetuses at 8 mg/kg bw/d, malformations were observed; these were considered to be incidental or secondary to maternal toxicity.

3.10.1.5 STUDY 3 - Developmental toxicity

Study reference: B.6.6.2, STUDY2

Characteristics

Type of study	:	Developmental toxicity	Exposure	:	orally by gavage; days 6-18 of gestation
Year of execution	:	1989-1992	Doses	:	0, 1.5, 3, and 6 mg/kg bw per day*
Test substance	:	MK 0243; L656,748-038W002 Benzoate salt Purity 94.2%	Vehicle	:	deionized water
Route	:	Oral	GLP statement	:	Yes
Species	:	Rabbit NZW	Guideline	:	-
Group size	:	18 pregnant females/dose	Acceptability	:	acceptable
			$NOAEL_{mat}$	:	3 mg/kg bw per day
			$NOAEL_{dev}$	:	6 mg/kg bw per day

* a factor 1.14 was used to calculated the dosages as base compound

Study design

Groups of 18 artificially inseminated young adult female rabbits were treated orally, by gavage, with L-656,748-038W (the benzoate salt) in water at dose levels of 0, 1.5, 3 and 6 mg/kg per day from days 6 through 18 of gestation. Clinical signs were recorded twice daily during the administration period (at dosing and 1-5 h after dosing) and daily thereafter. Body weights were recorded on days 0 and 28 and at 2-day intervals during the administration period and food consumption was measured every third day. Does were killed on day 28 of gestation and their pregnancy status established. Does were examined macroscopically, and the number of corpora lutea, the presence of resorptions and dead foetuses were examined. Foetuses were sexed, weighed, and examined for external alterations. All foetuses were given a visceral examination, and the heads from approximately one half of the foetuses in each litter were removed and processed for

evaluation of soft tissue alterations. All foetuses were processed and examined for skeletal malformations and variations.

Results

The results of the teratogenicity study are summarized in table 3.10.1.5-1.

Table 3.10.1.5-1 Results from a developmental toxicity study in rabbits.

	Dose (mg/kg bw per day)	**0**	**1.5**	**3**	**6**	**dr**
Maternal effects	Mortality	none				
	Clinical signs: - *alopecia* - *pulling fur* - *soft faeces* - *ocular discharge* - *increased urine* - *mydriasis* [a] - *decreased pupillary reaction* [b]	 0 1 6 1 1	 1 2 1	 4 4 1	 2 2 1 9 16	
	Pregnant animals	15	17	15	17	
	Abortions	1				
	Gravid uterine weight	not measured				
	Corpora lutea	Mean data not shown				
	Body weight changes [c]				dc [days 12-19] dc [days 6-28]	
	Food consumption				dc [day 10, 22, 25 and 28]	
	Water consumption	not measured				
	Pathology [d]	No treatment-related findings				
Litter response	Live foetuses	120	139	110	143	
	Foetal weight: m/f	35.1/33.4	36.3/35.0	38.1/37.6	36.1[ns]/ 34.1[ns]	
	% Pre implantation loss	9.8	15.0	14.5	10.5	
	No. of resorptions	3	4	4	1	
	Post implantation loss (% resorp+dead foet)/impl (litter mean))	3.5	3.1	4.1	1.9 [ns]	
	Sex ratio (m:f)	1 : 1.45	1 : 0.98	1 : 0.86	1 : 0.96	
Foetus examination	No. of live foetuses	120	139	110	143	
	No. of dead foetuses	0	0	1	2	
	External examinations	No findings				
	Visceral examinations					
	No. of foetuses with malformations	1	3	1	2	
	No. of malformations: - *ventricular septal defect* - *retrocaval ureter* - *epididymal malf.* - *hydrocephalus* - *cerebral malf.*	 1	 1 1 1	 1	 1 1	
	No. of foetuses with variations:	10	12	5	14	
	No. of variations: - *gall bladder reduced in size* - *variation in lung lobulation*	 1 10	 12	 5	 14	

	Dose (mg/kg bw per day)	0	1.5	3	6	dr
	Skeletal examinations					
	No. of foetuses with malformations	`2	2	2	5	
	No. of malformations: - *Thoracic vertebra malf.* - *lumbar vertebra malf.* - *caudal vertebra malf.* - *agenesis of rib* - *sternebral malf.* - *pelvic bone malf.*	 1 1 1	 2	 1 1 	 2 2 1	
	No. of foetuses with variations	2	3	0	5	
	No. of variations: - *skull bone var.* - *caudal vertebra var.* - *cervical rib* - *sternebral variation* - *pelvic bone var.*	 2 1 	 2 1	 	 1 2 2 1 	
	No. of foetuses with incomplete ossification:	33	34	21	36	
	No. of sites of incomplete ossification: [e]	43	43	25	40	

dc= decreased stat. significant; dr= dose releated; ns= not significant for trend test

a Mydriasis was observed between days 11 and 17 of gestation. In most affected animals, mydriasis was noted on less than 3 days and only as a post-dosing sign (not observed approximately 24 hours after the last dose). However, 2 affected females were still observed with mydriasis prior to dosing on days 2 and 6, respectively.

b 16 females in this group (including the 9 females showing mydriasis) were observed to have decreased pupillary reaction (slowed pupil constriction upon presentation of bright light) between days 11 and 23 of gestation. This physical sign appeared either prior to or after mydriasis and in most affected animals was observed approximately 24 hours after dosing.

c In the high dose group statistically significant decreases in body weight gain were seen during days 6-19 (40%) and during days 12-19 (52%). The overall reduction in body weight gain (days 6-28) was 29%.
In the mid dose group, reductions were observed during days 12-19 (10%) and days 19-28 (14%). The overall weight gain over the period days 6-19 was increased compared to control by 10%, and there was also no overall reduction in body weight gain over the total period (days 6-28). Therefore, the slight changes in weight gain in this dose group are not considered an adverse effect.

d In the uterus of one high dose doe unilateral hypoplasia was observed at the gross and microscopic examinations. This change was considered to have been in existence prior to the initiation of dosing and thus of no relation to compound administration.

e The main sites of incomplete ossification were: sternebra, metacarpal, forefoot phalanx, and pelvic bone.

No mortality occurred in the study. One doe of the control group had an abortion and was sacrificed. Treament-related clinical signs in does consisted of 9 does of the high dose group with mydriasis. All of these animals plus another seven also showed a slow pupillary reaction. A decrease in maternal weight gain (40% decrease) occurred in the high dose group, predominantly during the latter part of the dosing period. Food consumption in this group was slightly reduced (by up to 5%) on occasions during the dosing and post-dosing periods. No test substance-related gross or microscopic lesions occurred in maternal animals at any dose level.

There were no substance-related effects at any dose level on embryo survival and sex ratios (the control value for the sex ratio is unusual and the range in treated groups is 1:0.86 to 1:0.98 with no dose response). A slight increase in foetal weight seen in the mid dose group was not considered treatment-related since there was no dose relation. The external and visceral examinations revealed no substance-related effects. Slight increases in the number of skeletal malformations and variations were found in the high dose group, like malformations of lumbar vertebra and of sternebra and a slightly higher number of cervical rib (variant). According to the study authors the incidences of these anomalies were within the historical control data from the laboratory concerned. In table B.6.6.2-3 the incidences from the study and from the historical control data are shown. Although no extensive historical control data is available, the present reviewer can agree with the study authors that the increased incidences are not treatment-related.

Table 3.10.1.5-2 incidences of foetal anomalies from the study report and from the historical control data.

Foetal anomaly	**Foetal incidence in high dose group (study report)**	**Historical control incidence [1] (highest foetal incidence)**
lumbar vertebra	1.4%	2.38%
sternebral malformation	1.4%	4.55%
cerivical rib variation	1.4%	8.41%

[1] in the study report it is mentioned that the historical control data are from the performing laboratory since 1979. Only the highest incidence values were reported and there was no further data available.

Acceptability

The study was performed in accordance with the former OECD guideline 414 (before 2001) in which exposure during organogenesis only was prescribed. This deviation is considered acceptable.

Deviations: uterus weight was not determined.

Conclusions

Clinical signs of neurotoxicity were not observed in this study. The only clinical signs of maternal toxicity were mydriasis and decreased pupillary reaction in the high dose group and a decrease in body weight gain. The NOAEL for maternal toxicity is established at 3 mg/kg bw per day.

There was no evidence of a teratogenic effect in the rabbit, and no embryo and/or foetotoxic effects were observed at any dose level. Thus, the NOAEL for embryo/foetotoxicity is established at 6 mg/kg bw per day, the highest dose tested.

3.10.2 Developmental neurotoxicity

3.10.2.1 STUDY 1 - Developmental neurotoxicity

Study reference: B.6.7.3, STUDY 1

Characteristics

Type of study	:	Developmental neurotoxicity	Exposure	:	Orally by gavage; from day 6 of gestation through day 20 of lactation
Year of execution	:	1991-1993	Doses	:	0, 0.1, 0.6, and 3.6/2.5* mg/kg bw**
Test substance	:	MK-0244: L-656,748-052S002 Purity: >97%	Vehicle	:	Deionized water
Route	:	Oral	GLP statement	:	Yes
Species	:	Rat Crl:CD(SD) Br strain	Guideline	:	No guideline, but in accordance with OECD 426
Group size	:	25 f/dose	Acceptability	:	Acceptable
			NOAEL maternal:	:	2.5 mg/kg bw per day
			NOAEL developm. neurotox:	:	0.6 mg/kg bw mg/kg bw

* between gestation day 17 and 20 the high dose level of 3.6 mg/kg bw per day was reduced to 2.5 mg/kg bw per day due to the appearance of pup tremors in the 3.6 mg/kg dose group of a concurrent 2-generation reproduction study (see paragraph B.6.6.1)
** all dose levels are expressed as base compound (factor 1.15)

Study design

Groups of 25 inseminated young adult female rats were dosed orally, by gavage, from day 6 of gestation (day 0 = observation of vaginal plug) to day 20 of lactation with L 656,748-052S in water at dose levels of 0,

0.1, 0.6 or 3.6 mg/kg bw per day. The highest dose level was reduced to 2.5 mg/kg bw per day on days 17 - 20 of gestation due to adverse reactions noted in a parallel 2-generation toxicity study (TT #91-715-0, Lankas, G., (1993)). Maternal animals were examined daily from day 6 of gestation, body weights were recorded during gestation (every 2 days) and lactation (every 3 days), parturition was observed frequently and an accurate duration of gestation determined where possible. All maternal animals were killed at weaning, the uterus examined and the number of metrial glands counted. Pups were examined externally on the day of birth for abnormalities, sexed, and subsequently examined daily. Body weights were recorded on postnatal days 0, 4, 11, 17, and 21. Litter size was standardised to 4 pups/sex/litter on postnatal day 4. Culled pups were discarded without examination. Ten pups/sex/group were killed on postnatal day 11; brain and body weights were recorded. Six brains/sex/group were fixed and histopathology was performed on the brains of control and high dose group.

Behavioural testing, motor activity, auditory startle, learning ability and short-term memory function, were performed during lactation and long-term memory retention post-weaning. Pups were weaned on day 23/24 of lactation and checked for preputial separation or vaginal opening. Behavioural testing was repeated at 58 - 60 days of age following which the F1 generation animals were killed and at least 10 animals/sex/group subjected to necropsy limited to brain removal and weighing. A further 6 animals/sex/group were perfused and subjected to necropsy limited to removal of brain, spinal cord, optic and sciatic nerves and skeletal muscle. Sections of brain from 6 animals/sex killed at 11 and 60 days of age from the control and high dose groups were examined for histopathological alterations and histomorphometric measurements (of cerebral cortical depth, hippocampus major and single cerebellar folia) were recorded. In addition, sections of spinal cord, sciatic nerve, optic nerve and skeletal muscle from 6 animals/sex killed at 60 days of age from the control and high dose groups were examined for histopathological alterations. Body weight, reproductive and behavioural data were analysed statistically.

Results

The results are summarized in table 3.10.2.1-1.

Table 3.10.2.1-1: Results from a developmental neurotoxicity study in the rat

	Dose (mg/kg bw per day)	**0**	**0.1**	**0.6**	**3.6/2.5**	**dr**
Maternal effects						
	Mortality	none				
	Clinical signs:					
	- *alopecia*	6	5	6	2	
	- *slightly swollen hindpaws*	1				
	- *laceration*	1				
	- *trace blood in vagina*		1			
	- *regurgitatted part of dose*		1			
	- *nasal discharge*			1		
	- *lethargic/dystocia*			1		
	Pregnant animals	23	25	25	25	
	Females with no live pups			1		
	Abortions	none				
	Body weight changes during gestation [a]			ic [days 6-20]	ic [days 6-20]	dr
	Body weight changes during lactation	No treatment-related findings				
	Food consumption	not measured				
	Gestation length	No treatment-related findings				
	No. of implants per female	No treatment-related findings				
	Pathology	Not performed, not included in guideline				
Litter response						
	Live pups/litter day 0	14.8	15.8	15.0	15.5	
	Post implantation survival (% live pups on day 0/ no. of metrial glands) [b] (L.M.)	88.9	95.0	92.2	92.2	

	Dose (mg/kg bw per day)	**0**	**0.1**	**0.6**	**3.6/2.5**	**dr**
	% pup deaths (L.M.)					
	day 1-4	1.3	1.7	1.5	1.9	
	day 5-21	0.5	0.0	0.0	1.0	
	Sex ratio (m:f)	1 : 1.13	1 : 0.96	1 : 0.88	1 : 1	
Examination F1 pups						
	No. of live pups after culling	184	200	192	200	
	% pup deaths	No treatment-related findings				
	pup weight pre-weaning [c]				dc [m/f]	
	pup weight post-weaning [d]				dc [m/f]	
	Weight gain post weaning [e] week 1-7 f:					
	m:		-5%	-6% [dc]	-18% [dc]	
				-1%	-17% [dc]	
	External examinations (no of animals with:)					
	Hematoma (variation)		2			
	Clinical signs: pre-weaning (no. of animals/total examined) [f]					
	- intermittent head tremors				10/25	
	- intermittent body tremors				23/25	
	- whole body tremors				25/25	
	- hind limb extension				25/25	
	- hind limb splay				25/25	
	Clinical signs: post-weaning (no. of animals/total examined) [g]					
	- alopecia and/or scabs	1/161	1/175	3/169	4/174	
	- unkempt coat			1/169	6/174	
	- whole body tremors				7/174	
	- hind limb splay				79/174	
	- intermittent hind limb splay				1/174	
	- tail injury/tip of tail missing				2/174	
	Developmental landmarks [h]					
	- vaginal canalization (day)	33.7	33.4	33.1	37.4 [i]	
	- preputional separation (day)	44.8	44.9	44.8	48.4 [i]	
	Neurotox evaluation: pre-weaning					
	- motor activity [j]				ic [m/f]	
	- auditory startle response [k]				dc [m/f]	
	- passive avoidance [l]	No treatment-related findings				
	Neurotox evaluation: post-weaning					
	- motor activity [j]				dc [f] / d [m]	
	- auditory startle response [k]				dc [m/f]	
	- passive avoidance [l]	No treatment-related findings				
	Brain weights					
	- abs. brain weight (g) [n]					
	day 11 (m/f):	1.15/1.09	1.10/1.07	1.13/1.09	1.06/1.05	
	day 60 (m/f)	1.98/1.92	2.05/1.85	2.02/1.92	1.90 [dc] /1.73 [dc]	
	- rel. brain weight (%) [n]					
	day 11 (m/f):	4.06/3.99	4.07/4.14	3.92/4.16	4.49/4.24	
	day 60 (m/f)	0.50/0.76	0.50/0.73	0.52/0.76	0.60 [ic] /0.90 [ic]	
	Macroscopic examination	No treatment-related findings				
	Microscopic examination	No treatment-related findings				

ic= statistically significantly increased; i = increased; dc= statistically significantly decreased; d= decreased; dr= dose-related; L.M.= litter mean; m = male; f = female.

a Significant increases were observed for gestation days 6-20: 11% in the mid dose group and 15% in the high dose group.

b It is considered by the RMS that the post implantation survival based on metrial glands is comparable to the post implantation survival based on implantation sites (which is normal practice). The metrial gland is a gland structure in the uterus.

c Female and male pup weights were decreased on postnatal days 11, 17 and 21. The decrease was highest on day 21 and was about 42% in females and 40% in males compared to controls.

d Male and female pup weights were decreased in lactation week 7 (22% for males and 25% for females).
e A significant decrease in pupweight gain over lactation period week 1-7 was observed in males and females of the high dose group. Also in females of the middose group a slight but statistically significant decrease was observed. However, this decrease was only 6% and a similar (non-significant) decrease was observed in the low dose females. Therefore the decrease in weight gain in the female middose group is not considered biologically significant.
f In the table only the treatment-related signs are mentioned. Intermittent head and body tremors were observed between post natal days 6-10 and days 7-13, respectively. Whole body tremors and hindlimb extension were observed during days 10-25 and hindlimb splay during days 15-26.
g In the table only the treatment-related signs are mentioned. Alopecia was observed during days 39-41 and 58-68; unkempt coat during days 37-41; (Intermittent) hind limb splay during days 24-34; whole body tremors on days 24 and 27; and the tail injury was observed from day 34.
h Expressed as mean day (post natal) of occurrence.
j Horizontal activity was assessed during pre-weaning on postnatal days 13, 17 and 21 and post weaning on day 59-60. The activity was increased in both sexes on postnatal day 13 (70% (f) and 54% (m)). At day 17, the activity was decreased (41% (f); 30% (m)) and was slightly increased again at day 21. The activiy was significantly decreased in females on postnatal day 58 (18%) and not significantly decreased in males (8%).
k Auditory startle response was evaluated on postnatal days 22 and 58-60. The amplitude of the response was reduced on day 22 in both sexes by 74%, and the interval between stimulus and peak response was increased by 22% in females and 33% in males. On post natal day 58-60 the amplitude of the response was still reduced in both sexes (32% (m); 46% (f)).
l The passive avoidance test is a learning and memory test and was performed for preweaning animals on day 24 (learning and short term retention) and day 31 (long term retention) and for post-weaning animals on days 59 and 66.
n In both 11- and 60 day old pups, absolute brain weights in the high dose group tended to be slightly lower than controls, but were considered to be secondary to the observed growth retardation since brain/body weight ratios were increased (significantly in 60 day old pups).

No deaths or abortions occurred and there were no treatment-related clinical signs of toxicity in F0 females at any dose level, but weight gain during gestation was significantly ($p < 0.05$) elevated by 11 and 15% in the 0.6 and 3.6/2.5 mg/kg bw per day groups, respectively. This increase in body weight gain is not considered adverse, given the absence of any other effect in these females.

Reproductive performance, as assessed by implantation rate, live litters, duration of gestation, post-implantation survival and pup viability at birth, was unaffected at all dose levels. Further, no effects were found on the external morphology of F1 pups and their sex ratio and pre-weaning survival.

In pups of the high dose group, intermittent head tremors progressing to whole body tremors and hind limb extension progressing to hind limb splay were observed in all pups, starting between day 6 and day 10 of lactation and persisting into the post-weaning period. Pre-weaning and post-weaning pup weights and weight gain were reduced in both sexes at this dose level. A delay of 3.6 - 3.7 days in preputial separation and vaginal opening, also observed in pups of the high dose group are considered to be associated with this reduced weight gain.

Test substance-related behavioural effects were observed in pups of the high dose group, but not at lower dose levels. Increased motor activity, occurring on day 13, is interpreted by the study authors as an expression of stereotypical behavioural movement consistent with the observed tremors. On day 17, motor activity was reduced by 30 – 41% but was not significantly affected on day 21. An effect on the auditory startle response was apparent on day 22, the amplitude of response was reduced by 74% and the interval between stimulus and peak response increased by 22 – 33%. Effects on motor activity in females and the auditory startle response in both sexes persisted into the post-weaning period. Learning and short- and long-term retention, measured by a passive avoidance technique, was unaffected by test substance at all dose levels.

The effects on brain weights seen in both 11 and 60 day old pups of the high dose group were considered to be secondary to the observed growth retardation.

No test substance-related histopathological and histomorphometric effects occurred in the brains of either the 11 or 60 day old F1 pups. Further, no test substance-related gross or microscopic alterations occurred in central and peripheral nervous tissues or skeletal muscle in 60 day old F1 pups from the high dose group.

Acceptability

The study is in accordance with the draft guideline OECD 426 except that food consumption was not measured.

The study is considered acceptable.

Conclusions

The NOAEL for emamectin benzoate hydrate for developmental neurotoxicity was established as 0.6 mg/kg bw per day, based on the occurrence of clinical evidence of neurotoxicity, growth retardation and alterations of neurobehavioural function in the F1 progeny of females administered emamectin at 2.5 mg/kg bw per day during the period of gestation (day 6) through lactation (day 20). In the pups, no histopathological changes was observed.

In the absence of evidence of toxic effects in dams, the NOAEL for maternal toxicity was 2.5 mg/kg bw/day, the highest dose tested.

Remark:

To convert the dose to base compound a factor 1.15 was used instead of 1.16.

3.10.3 Human data

No data available.

3.10.4 Other data (e.g. studies on mechanism of action)

No data available.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

Please refer to section 3.1-3.3.

3.11.2 Human data

No data available.

3.11.3 Other data

No data available.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 STUDY 1 - 13-week toxicity study in rat

Study reference: B.6.3.3, STUDY 1

Characteristics

Type of study	:	13-week toxicity study in rat	Exposure	:	Repeated by diet, 13 weeks
Year of execution	:	1988-1992	Doses[a]	:	0, 0.5, 2.5, 12.5/8/5 mg/kg bw/day
Test substance[a]	:	L-656,748-010V003 (purity 92.8% B1a and 4.1% B1b)	Vehicle	:	-
Route	:	oral	GLP statement	:	yes
Species	:	Rat Crl:CD[SD] BR	Guideline	:	OECD 408
Group size	:	20/sex/group	Acceptability	:	acceptable
			NOAEL	:	0.5 mg/kg bw/day

a: the bulk drug contained 0.76 weight % propylgallate as an antioxidant; a factor of 1.04 was used for calculation of the desired concentrations.

Study design

Rats (20/sex/dose) were given 0, 5, 25 or 150 ppm (0, 0.5, 2.5 or 12.5 mg/kg bw/day) emamectin for 13 weeks in their diet (based on a 3-weeks range finding study with rats, see B6.3.1. study 1). Due to decreases in food consumption and bw gain, the dose of 12.5 mg/kg bw/day was reduced to 8 mg/kg bw/day (55-94 ppm) during weeks 3 to 7, and subsequently to 5 mg/kg bw/day (about 60 ppm) during weeks 9 to14. The study was performed according to former OECD guideline 408, except for conduct of FOB (not required then) and histopathological examination of the epididymides. Full histopathology was confined to controls and high dose animals. Bone, skeletal muscle, brain, spinal cord, sciatic and optic nerve and gross lesions were examined in rats from all dose groups. Statistical analysis were not performed.

Results

Table 3.12.1-.1-1 Results from a 13-week oral toxicity study in rats

Dose (mg/kg bw/day)	**0**		**0.5**		**2.5**		**12.5/8/5**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality[a]							9/20		
Clinical signs -fine tremors -splaying and limited use hindlimbs -genital area stained by urine							 20/20 8/20 10/20	 20/20 5/20 2/20	
Body weight gain (g) -compared to controls	360	173	374	153	356	182	181 -36%	86 -31%	
Food consumption (g/day) -compared to controls	24.5	18.5	26.4	17.5	25.5	19.1	20.3 -20%	15.4 -17%	
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology[b] -erythrocyte (million/mm^3) -Hb (g/100 mL) -Ht (%) -lymphocytes (cells/mm^3) -leucocytes (1000/mm^3) -segm. neutrophils (cells/mm^3) -monocytes (cells/mm^3)	 7.9 15.7 43 12129 14.4 1426	 955	 8.0 15.6 43 11127 13.3 1418	 948	 7.9 15.6 43 11494 13.8 1520	 967	 8.3 16.1 45 9819 11.7 1281	 756	

Dose (mg/kg bw/day)	**0**		**0.5**		**2.5**		**12.5/8/5**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
	607	322	504	319	580	272	434	244	f
Clinical Chemistry[b]									
-glucose (mg/dL)	128	126	127	113	120	131	94	92	
-urea nitrogen (mg/dL)	14.0	14.5	14.5	15.1	14.0	15.2	15.2	16.8	
Urinalysis[b]									
-volume	24.2	13.3	22.3	12.4	19.7	15.5	10.1	6.7	
-spec. gravity	1.017	1.023	1.020	1.023	1.022	1.017	1.032	1.037	
Organ weights (%bw)									
-spleen	0.16	0.18	0.16	0.19	0.14	0.18	0.18	0.23	
-kidneys	0.77	0.79	0.75	0.80	0.79	0.77	1.06	1.04	
-liver	2.81	2.78	2.82	2.96	2.89	2.86	3.48	3.64	
-adrenals	0.012	0.026	0.012	0.029	0.013	0.026	0.019	0.035	
-lungs	0.34	0.45	0.35	0.48	0.35	0.47	0.47	0.57	
-thyroid	0.0043	0.0059	0.0045	0.0065	0.0044	0.0055	0.0059	0.0080	
-pituitary	0.0028	0.0056	0.0027	0.0056	0.0029	0.0053	0.0039	0.0073	
-thymus	0.06	0.11	0.06	0.10	0.06	0.10	0.08	0.13	
-testes	0.70		0.68		0.70		1.08		
-ovaries		0.029		0.032		0.029		0.040	
-uterus		0.26		0.25		0.19		0.37	
Pathology									
Macroscopy									
- emaciation	0	0	0	0	0	0	20	20	
- decreased muscle mass (especially hind leg)	0	0	0	0	0	0	20	20	
microscopy									
- brain, neuron vacuolation	0	0	0	0	2	0	15	16	m
- spinal cord, neuron vacuolation									
- spinal cord, degeneration	0	0	0	0	0	0	20	18	
- sciatic nerve, degeneration									
- skeletal muscle, atrophy	0	0	0	0	0	0	10	5	
- eye, degeneration optic nerve									
- bone, trabecula atrophy	0	0	0	0	0	0	17	18	
	0	0	0	0	0	0	20	20	
	0	0	0	0	1	0	0	1	
	0	0	0	0	0	0	17	9	

a: death of 1 control and 1 mid-dose male, and 1 control, 1 low-dose and 2 high-dose females were not substance-related.

b: mean value of the investigated time points

In weeks 3 to 11, nine high-dose males were euthanized due to emaciation and/or poor appearance asociated with decreased food consumption and body weight gain. Treatment-related tremors were observed in high-dosed animals throughout the study, while hindlimb splaying was first observed in week 7. Reduced body weight gain and food consumption were observed throughout the study period in both sexes of the highest dose group. Effects on haematological and clinical chemistry parameters were observed in the highest dose group, including effects on plasma glucose level and on numbers of leucocytes, lymphocytes, monocytes and segmented neutrophils. Increased relative organ weights (113-158% compared to controls) were observed in both males and females of the highest dose group for a number of organs, which may be related to the observed decreased bw gain in the highest dose group. Observed gross pathological findings were confined to the highest dose group (observed both in rats dying during the study and in survivors), and consisted of emaciation and decreased muscle mass (especially evident in the hind leg). Microscopically, changes were observed in the brain, spinal cord, sciatic and optic nerves, skeletal muscle and bone of rats from the highest

dose group, and in the brain from 2 males in the mid-dose group. The observed microscopic changes were very slight white matter degeneration and vacuolation of the neuronal cytoplasm in the brain, spinal cord and peripheral nerves, together with neurogenic atrophy of skeletal muscle. Besides, trabecular atrophy of the femur was observed in these animals.

Acceptability

The study is considered acceptable for the overall toxicological evaluation.

Conclusions

Administration of emamectin at a dose level of 12.5 mg/kg bw/day by diet during 13 weeks resulted in tremors and a marked decrease in weight gain in rats, together with effects on blood glucose and numbers of leucocytes, lymphocytes, monocytes and segmented neutrophils. Reduction of the dose level to 8.0 and then to 5.0 mg/kg bw/day during week 3 and 8 resulted in some improvement, although the signs of toxicity persisted in most of the affected animals. The observed signs of neurotoxicity in rats of the highest dose group were accompanied by morphological changes in the brain, spinal cord, optic and sciatic nerves, and bone and skeletal muscle. Although animals at the mid dose (2.5 mg/kg bw/day) did not display signs of neurotoxicity, 2 males had similar lesions in the brain as animals in the high dose group. Therefore, the NOAEL in this study is 0.5 mg/kg bw/day.

3.12.1.2 STUDY 2 - 13-week toxicity study in mice

Study reference: B.6.3.3, STUDY 2

Characteristics

Type of study	:	13-week toxicity study in mice	Exposure	:	Repeated by diet, 13 weeks
Year of execution	:	1990-1992	Doses	:	0, 0.5, 1.5/10, 4.5 and 15 mg/kg bw/day
Test substance[a]	:	MK-0243 (L-656,748-038W002, benzoate salt; purity 91.1% B1a and 5.1% B1b)	Vehicle	:	-
Route	:	Oral	GLP statement	:	yes
Species	:	Mouse (Crl:CD-1 (ICR) BR strain	Guideline	:	-
Group size	:	15/sex/dose	Acceptability	:	Acceptable as range-finding study for carcinogenicity study
			NOAEL	:	-

a: a factor of 1.14 was used.for calculation of the desired concentrations since MK-0243 was provided as a benzoate salt.

Study design

Mice were fed diets containing 0, 0.5, 1.5, 4.5 or 15 mg/kg bw/day (0, 3, 9, 27 or 90 ppm) L-656,748-038W (benzoate salt) during 84 days. In week 8 the dose administered to the 1.5 mg/kg bw/day group was increased to 10 mg/kg bw/day (60 ppm) L-656,748-038W in order to better define the toxicity of the test substance at a dose level between 4.5 and 15 mg/kg bw/day. No information was provided on housing conditions. Mice were observed daily for mortality and clinical signs, and weighed weekly. Food consumption was measured over a six-day interval weekly. Ophthalmoscopic examination was performed prior to the study and with control and high dose animals in week 12. At scheduled termination of the study blood was collected from fasted animals for haematological examination (erythrocyte count, Hb, Ht, MCV, MCH, MCHC, (differential) leukocyte count, platelet count) and serum biochemical determination (protein, albumin, A/G ratio, glucose, urea nitrogen, creatinine, bilirubin, AST, ALT, AP, cholesterol, triglycerides, sodium, potassium, chloride, calcium, phosphorus).

Microscopic examination was confined to 5 control and 5 high dose animals.

Results

One male and one female in the highest dose group died. Neither clinical signs nor histopathological changes were observed in these animals. There were no clinical signs observed at any dose level. In the highest dose group, reduced body weight gain was observed in both males (-41%) and females (-21%), which was not clearly related to a decrease in food consumption. Although some haematological parameters were different in the high-dosed animals compared to controls, the observed changes were small and not consistent between males and females. Therefore, these differences are considered not toxicologically relevant. Clinical chemical investigation of blood showed decreased plasma glucose values in males and females of the highest dose group and in males of the 4.5 mg/kg bw/day group. The values of the 1.5/10 mg/kg bw/day group are considered not representative for 13 weeks exposure to a defined dose level. In males of the highest dose group, a 16% increase of relative (to bw) liver weight was observed. No histomorphological alterations were observed in the 5 animals of the highest dose group examined.

Acceptability

Since the dose of the 1.5 mg/kg bw/day group was increased to 10 mg/kg bw/day during weeks 8-13, data from this group cannot be considered as exposure to 10 mg/kg bw/day during 13 weeks. Microscopic examination was confined to 5 control and 5 high dose animals.

No data on ophthalmoscopic examination and on plasma Na, Ca, Cl, P, K, ALP, cholesterol, bilirubin and triglycerides were included in the study report. In the study it is reported that the concentration analysis of the test diet showed an average % nominal study sample of 85.4 % with a 3.5% coefficient of variation (n=56). From the presented study results it is unclear whether the reported dose concentrations are corrected by this factor.

Since this study was performed as range-finding study for a carcinogenicity study in mice with MK-0243, the information from this study can be used for that purpose.

Conclusions

Based on the results of this study, a maximum level of approximately 12.5 mg/kg bw/day was chosen for a subsequent carcinogenicity study in mice with MK-0243.

3.12.1.3 STUDY 3 - 52-week toxicity study in rats

Study reference: B.6.3.3, STUDY 3

Characteristics

Type of study	:	52-week toxicity study in rats	Exposure	:	Repeated by diet, 52 weeks
Year of execution	:	1991-1992	Doses[b]	:	m: 0, 0.1, 1.0 and 2.5 mg/kg bw/day f: 0, 0.1, 1.0 and 5.0/2.5 mg/kg bw/day
Test substance[a]	:	MK-0244 (L656,748-052S002; 97.2-97.8% area percent (95.9 weight%) pure (92.5% B1a, 5.3% B1b)	Vehicle	:	-
Route	:	oral	GLP statement	:	Yes, with deviations
Species	:	Rat (Crl:CD(SD) BR)	Guideline	:	-
Group size	:	20/sex/dose	Acceptability	:	acceptable
			NOAEL	:	1.0 mg/kg bw/day

a: a factor of 1.15 was used for all dosage calculations

b: overall ranges of nominal concentrations for each dose level were 83.7-107.1%, 77.2-96.2 and 81.1-95.5% for males in the 0.1, 1.0 and 2.5 mg/kg bw/day groups, respectively and 90.5-111.1%, 85.9-104.1% and 91.1-102.7% for females in the 0.1, 1.0 and 5.0/2.5 mg/kg bw/day groups, respectively.

Study design

Groups of rats (20/sex) were orally administrated L-656,748-052S (benzoate hydrate salt) by diet for 52 weeks at dose levels of 0, 0.1, 1.0 or 2.5 mg/kg bw/day. High dose females received 5.0 mg/kg bw/day for the first 18 weeks and 2.5 mg/kg bw/day thereafter (overall mean concentration of 3.4 mg/kg bw/day). Housing conditions were not reported. Clinical observations were made daily, and body weights and food consumption were recorded weekly. Haematology, clinical biochemistry and urinalysis were performed on 10 rats/sex/group in weeks 13, 26 and 52, and ophthalmoscopic examinations were performed pre-test and in week 50. Functional observation battery (FOB) and motor activity tests were performed with 10 rats/sex/group in weeks 13, 24, 38 and 51. At the scheduled termination of the study, animals were subjected to gross pathology and organ weights were recorded from adrenals, brain, kidneys, liver and testes. Histopathology was performed on all control and high dose animals and on animals dying during the study. Brain, spinal cord, liver, kidney, lung and any grossly abnormal tissues were examined microscopically from all animals. A trend analysis was done on FOB and motor activity data.

Results

Table 3.12.1.3-1 Results from a 52-week oral toxicity study in rats

Dose (mg/kg bw/day)	**0**		**0.1**		**1.0**		**2.5**	**5.0/2.5**	**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	Not treatment-related								
Clinical signs -fine tremors[a] -unkempt appearance[a] -urine staining[a]								 9/20 9/20 9/20	
Body weight gain (g) weeks 1-53	568	263	590	265	597	301	610	294	
Mean food consumption (g/day)	No toxicologically relevant effects								
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology	No toxicologically relevant effects								
Clinical Chemistry, week 52 -glucose (mg/dL) -total cholesterol (mg/dL) -triglycerides (mg/dL)	 90 133	 143 	 132 210	 151 	 123 220	 150 	 121 298	 158 	
Urinalysis - mean volume week 13, 26, 52 (mL)	6.5		7.0		7.8		8.1		m
FOB -open field, arousal normal (week 51) -grip strength forelimb (% of control; week 14)	8/10 	 100%	4/8 	 105%	5/10 	 103%	1/10 	 70%	
Organ weights (%bw) -testes (absolute, g) -testes (% bw)	 3.88 0.55		 4.01 0.53		 3.73 0.47		 3.63 0.44		 m m
Pathology									
Macroscopy	No toxicologically relevant effects								
Microscopy -liver, centrilobular vacuolation -liver, periportal vacuolation - brain, neuron degeneration - spinal cord, neuron degeneration	 0/20 3/20 0/20 0/20	 2/20 1/20 0/20 0/20	 0/20 2/20 0/20 0/20	 2/20 1/20 0/20 0/20	 1/20 3/20 0/20 0/20	 1/20 7/20 0/20 0/20	 3/20 3/20 9/20 4/20	 6/20 6/20 19/20 2/20	

Dose (mg/kg bw/day)	**0**		**0.1**		**1.0**		**2.5**	**5.0/2.5**	**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	

a: tremors were observed in 1/20 females in week 9 to 9/20 females in week 18. After week 21 no more tremors were observed in high-dose females. Many of the animals showing tremors also had occasionally urine staining and unkempt appearance, and tremors were generally associated with reduced weight gain or weight loss.

Fine, whole body tremors, unkempt appearance and urine staining were observed in females in the 5.0 mg/kg bw/day group from week 3 until three weeks after dose reduction in week 18. Average body weights of high dose females were about 6% lower during weeks 16-23. Thereafter, weight gain increased, resulting in about 12% greater final body weight gain compared to controls. Higher final body weight gain was also observed in mid dose females (14%), however without dose-response. In male rats, plasma triglyceride levels were increased in all dose groups by 158-224% compared to control values (together with small (4-7%) increases in overall bodyweight gain). However, since the dispersion in plasma triglyceride levels in the low and mid dose groups was high, with values within historical control values for plasma triglyceride and no dose response between low and mid dose, only the increase in high dose males (with values outside historical control values) is considered toxicologically relevant. Compared to control values, higher mean urine volume were observed in male rats of the two highest dose groups in weeks 13, 26 and 52. This observation is in contrast with the observed decreased urine volume in the 13-week toxicity study in rat (see B6.3.3 study 1). In that study, control volumes were much higher compared to the control volumes in the present study (25.2 mL vs 6.5 mL for males and 13.3 mL vs 5.5 mL for females, repectively).

Microscopic examination of tissues and organs showed in females an increased incidence of liver centrilobular vacuolation at the high dose and of liver periportal vacuolation at the mid and high dose. The latter was, however, without dose-relationship. Neuronal degeneration in the brain and the spinal cord were observed in both sexes of the highest dose group.

Open field examination showed a decreased number of male rats with normal arousal in all exposed groups, which was statistically significant in the highest dose group only. Affected animals showed a shift to a slightly lower or lower arousal than normal. A decrease in forelimb force strength (to 70% of control value) was observed in females of the highest dose group in week 14. In week 24, forelimb force strength was 90% of control value, and in weeks 38 and 51 no difference with control values was observed.

Table 3.12.1.3-2 Statistical analysis of FOB.

category	sex	Week 0	Week 14	Week 24	Week 38	Week 51
Autonomic	Male	-	-	-	high	high
	Female	High^{N}	-	-	-	-
Muscle tone and equilibrium	Male	-	High Mid low	High mid	High Mid low	High Mid low
	Female	-	-	-	-	High^{N}
Sensor motor responses	Male	-	-	-	-	-
	female	-	-	-	-	-
Central nervous system	Male	-	-	-	-	high
	Female	-	-	-	-	-
No. of light beams broken	Male	-	-	-	-	-
	Female	-	-	-	-	-

High doe: 0.1 mg/kg day; mid dose 1.0 mg/kg day; low dose 5.0/2.5 mg/kg day

Significant dose effects are < 0.05, 1-sided, N negative trend

Acceptability

In spite of the GLP-deviations and different exposure scenario's for high dose males en females, the study is considered acceptable.

Conclusions

Clinical signs of neurotoxicity (f) and neuronal degeneration in brain and spinal cord were observed in high dose rats. At this high dose level, males showed increased plasma triglyceride levels, together with a small increase in overall bodyweight gain, and a shift to lower arousal than normal; females showed increased plasma glucose levels, decrease in grip strength of the forelimb (in week 14 only) and an increase in centrilobular vacuolation in the liver. Based on these effects in the high dose group, the NOAEL in this study is 1.0 mg/kg bw/dag.

3.12.1.4 STUDY 4 - 14-week toxicity study in dogs

Study reference: B.6.3.3, STUDY 4

Characteristics

Type of study	:	14-week toxicity study in dogs	Exposure	:	Repeated by gavage, 14 weeks
Year of execution	:	1988-1992	Doses[a]	:	Days1-14/15: 0, 0.5, 1.0 and 1.5 mg/kg bw/day Days 14/15-91/92: 0, 0.25, 0.5 and 1.0 mg/kg bw/day
Test substance	:	L656,748-010V003 (hydrochloride salt; purity 96.9%)	Vehicle	:	water
Route	:	oral	GLP statement	:	Yes[b]
Species	:	Beagle dog	Guideline	:	According to OECD 409
Group size	:	4/sex/dose	Acceptability	:	acceptable
			NOAEL	:	0.25 mg/kg bw/day

a: a factor of 1.04 was used for all dosage calculations. the test material contained 0.76% (w/w) propylgallate as an antioxidant.

b: GLP statement was signed in 1994, whereas the study was performed between 1988 and 1992; included QA inspections were signed in 1988 only.

Study design

Groups of dogs (5/sex) were orally administrated L-656,748-010V003 (hydrochloride salt) by gavage for 91/92 days at dose levels of 0, 0.5, 1.0 or 1.5 mg/kg bw/day. The dose levels were reduced to 0, 0.25, 0.5 and 1.0 mg/kg bw/day on day 14/15. Selected animals in the high dose group (with poor food consumption of the regular pelletted food) occasionally received special food. The study was performed according to OECD guideline 409. In addition, electrocardiograms were recorded from all dogs prior to the study and in weeks 4, 8 and 12 approximately 3 to 5 hours following dosing.

Results

Table 3.12.1.4-1 Results from a 14-week oral toxicity study in dogs

Dose (mg/kg bw/day)	**0**		**0.5/0.25**		**1.0/0.5**		**1.5/1.0**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality							1/4	2/4	
Clinical signs -tremors -mydriasis -ptyalism -recumbency -ataxia							 3/4 1/4 1/4 2/4	 3/4 1/4 2/4 3/4	
Body weight gain (g)	2.5		2.3		2.4		1.7		
Mean food consumption days 9-18 (% of control)							42-70%		

Dose (mg/kg bw/day)	**0**		**0.5/0.25**		**1.0/0.5**		**1.5/1.0**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Water consumption	Not recorded								
Ophthalmoscopy	No toxicologically relevant effects								
Electrocardiography	No toxicologically relevant effects								
Haematology	No toxicologically relevant effects								
Clinical Chemistry -urea nitrogen (mg/dL)		16.9		17.2		16.6		12.6	
Urinalysis	No toxicologically relevant effects								
Organ weights	No toxicologically relevant effects								
Pathology									
Macroscopy	No toxicologically relevant effects								
Microscopy - brain, neuron degeneration - brain, white matter, multifocal degeneration - spinal cord, multifocal degeneration - nerve degeneration - eye, optic nerve degeneration - skeletal muscle atrophy - thymus atrophy - bone marrow, decreased number erythropoietic tissue					 3/4 1/4 1/4	 1/4 1/4	 3/4 3/4 4/4 4/4 2/4 3/4 1/4	 3/4 3/4 4/4 3/4 3/4 4/4 2/4	 f m m,f

Two dogs (1m and 1f) dosed 1.5 mg/kg bw/day were killed *in extremis* in week 2 and another female dog dosed at the reduced level of 1.0 mg/kg bw/day was killed in week 6. Prior to death, the animals were observed with tremors, mydriasis, anorexia, lethargy and recumbency. Surviving dogs at the highest dose level showed ataxia and fine whole-body tremors. Decreased bodyweight in animals of the highest dose group were observed in weeks 2 and 3, together with decreased food consumption during days 9-18 in this dose group. Reduction of the dose level from 1.5 to 1.0 mg/kg bw/day resulted in improved bodyweight gain and food consumption in all but one dog in this group (which was killed in week 6) to weight gain and food consumption comparable to the controls. However, selected animals in the high dose group (with poor food consumption of the regular pelletted food) occasionally received special food. Therefore, data on food consumption and bodyweight gain in this dose group are not representative for the actual effects of exposure to the substance.

Microscopic changes were observed in the brain, spinal cord, peripheral and optic nerves, skeletal muscle, thymus and bone marrow of dogs in the highest dose group. The observed slight to severe decrease in the number of erythropoietic cells and thymus atrophy were confined to the animals that were killed during the study. White matter degeneration in the brain, degeneration of the spinal cord and atrophy of skeletal muscle were also observed in dogs from dose group 1.0/0.5 mg/kg bw/day.

Acceptability

Since selected animals in the high dose group (with poor food consumption of the regular pelletted food) occasionally received special food, the observed effects of the substance on food consumption and bodyweight gain in the highest dose group are not representative for the test substance in this dose group.

The study is considered acceptable for the overall toxicological evaluation.

Conclusions

Administration of the test substance to dogs resulted in clinical signs of neurotoxicity, decreased food consumption and body weight loss at a dose level of 1.5 mg/kg bw/day (highest dose group) after approximately two weeks. Reduction of the dose from 1.5 to 1.0 mg/kg bw/day resulted in less severe signs of neurotoxicity. Based on the observed morphological lesions in the brain, spinal cord, and skeletal muscle at the mid and high dose, the NOAEL in this study is 0.25 mg/kg bw/day.

3.12.1.5 STUDY 5 - 52-week toxicity study in dogs

Study reference: B.6.3.3, STUDY 5

Characteristics

Type of study	:	52-week toxicity study in dogs	Exposure	:	Repeated by gavage, 52 weeks
Year of execution	:	1990-1992	Doses	:	0, 0.25, 0.5 and 1.0 mg/kg bw/day additional group of 0.75 mg/kg bw/day
Test substance[a]	:	MK-0244 (L656,748-038W002; purity >97%) benzoate salt	Vehicle	:	Distilled water
Route	:	oral	GLP statement	:	yes
Species	:	Beagle dog	Guideline	:	OECD 452
Group size	:	4/sex/dose	Acceptability	:	acceptable
			NOAEL	:	0.25 mg/kg bw/day

a: a factor of 1.14 was used for all dosage calculations

Study design

Groups of dogs (4/sex) were orally administrated L-656,748-038W (benzoate salt) by gavage during 52 weeks at dose levels of 0, 0.25, 0.5 or 1.0 mg/kg bw/day. Because of signs of overt toxicity in all animals of the highest dose group, all dogs were sacrificed (without blood sampling) after 19 consecutive doses and 4 days without treatment. An additional group of 4 dogs/sex was added, at a dose level of 0.75 mg/kg bw/day. Because of signs of overt toxicity all males from this group were sacrificed after 49 doses. The study was performed according to OECD guideline 452. Full histopathology was performed on all control, 0.75 and 1.0 mg/kg bw/day animals and male 0.5 mg/kg bw/day animals. Gross lesions and muscle, brain, spinal cord, peripheral nerves and eyes were examined in all study animals. Statistical analysis were not performed.

Results

Table 3.12.1.5-1 Results from a 52-week oral toxicity study in dogs

Dose (mg/kg bw/day)	**0**		**0.25**		**0.5**		**0.75**		**1.0**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality							4/4		4/4	4/4	
Clinical signs											
-fine tremors						1/4	4/4	3/4	4/4	4/4	f
-mydriasis							1/4	1/4	3/4	4/4	m,f
-decreased motor activity									2/4	4/4	
-stiffness hindlegs						1/4	2/4	2/4			f
-difficult to get up								2/4			
-ataxia								1/4			
-hyperreaction to touch								1/4			
Body weight gain (g)							d		d	d	
Mean food consumption (g/day)[a]										d	

Dose (mg/kg bw/day)	**0**		**0.25**		**0.5**		**0.75**		**1.0**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Water consumption	Not performed										
Ophthalmoscopy[b]	No toxicologically relevant effects										
Haematology, week 52											
-prothrombine time	6.6[c]							5.9			
(sec)	314							391			
-platelets	11.5							8.3			
(1000/mm^3)	7362		7061		6332			5458			dr
-leucocytes	548[d]							194			
(1000/mm^3)											
-neutrophils											
(cells/mm^3)											
-eosinophils											
(cells/mm^3)											
Clinical Chemistry, week 52											
-glucose (mg/dL)	108		105		104			73			
-urea nitrogen	14.5							12.3			
(mg/100 mL)											
LDH (u/L)	76							130			
Urinalysis	No toxicologically relevant effects										
Organ weights	No toxicologically relevant effects										
Pathology											
Macroscopy	No toxicologically relevant effects										
Microscopy											
-skeletal muscle,						1/4	1/4	3/4			f
fiber focal											m,f
degeneration					1/4	2/4	4/4	3/4	4/4	4/4	
- brain, axonal											
degeneration							2/4		1/4	2/4	
-brain, neuron focal											
degeneration						2/4	4/4	2/4	4/4	4/4	
- spinal cord, axonal											
degeneration					3/4	1/4	4/4	4/4	4/4	4/4	m,f
-nerve, axonal											
degeneration							2/4	1/4	3/4	3/4	m,f
-eye, retina cellular											
degeneration							3/4	1/4	4/4	3/4	m,f
-eye, optic nerve											
axonal degeneration											

a: in week 47 and weeks 49-52 food consumption data from the 0.25 mg/kg bw/day group were provided for 4f and 2m only.

b: no data were provided, only described in result section of the study.

c: pretest value from the 0.75 mg/kg bw /day group was higher than pretest control value. During the study, control values were as high or higher than the pretest value, whereas lower values were observed in dogs from the 0.75 mg/kg bw/day group from week 9 on.

d: pretest values were comparable for control (284 cells/mm3) and 0.75 mg/kg bw/day animals (274 cells/mm3). At week 52, control values were increased to 548 cells/mm3.

In the 1.0 mg/kg bw/day group all animals showed signs of severe toxicity, and were killed after 19 doses. In the 0.75 mg/kg bw/day males, physical signs appeared from week 5 and consisted of fine whole body tremors, mydriasis and stiffness of hind legs. Because of these signs of overt toxicity, all males in this group were killed after 49 doses. Females in this dose group showed fine whole body tremors and mydriasis from week 5 onwards, and from week 22 more clinical signs of toxicity were apparent. One female in the 0.5

mg/kg bw/day showed fine whole body tremors from week 15 onwards and stiffness of the hindlegs in weeks 32-35.

In the 1.0 mg/kg bw/day group, weight loss was observed in 3 females (0.3-1.5 kg), accompanied by decreased food consumption, and in 1 male (0.6 kg). In the 0.75 mg/kg bw/day group 3 males lost weight (0.4-1.5 kg).

In high dose animals effects on blood glucose level and leucocyte number was observed, whereas a dose-related decrease in neutrophil numbers was observed at and above 0.5 mg/kg bw/day.

Histological examination showed substance-related changes in the central and peripheral nervous system at and above 0.5 mg/kg bw/day, and in the eye at and above 0.75 mg/kg bw/day. Muscle fibre degeneration was observed in the skeletal muscle of 1 male and 3 females in the 0.75 mg/kg bw/day group and in 1 female of the 0.5 mg/kg bw/day group.

Acceptability

The bodyweight of the dogs at the start varied considerably (f: 6.5 to 12.1 kg, m: 8.7-12.2 kg). No data on ophthalmologic examination were included in the study report. Blood samples for haematological and clinical biochemical analysis from dogs of the 0.75 mg/kg bw/day group were taken at different time points (week 7, 9, 21, 48 and 52) during the study compared to dogs from the other dose groups (week 13, 25 and 52). The analysis from the 0.75 mg/kg bw/day group was not accompanied by values from control animals. Therefore, in that group results were compared to pretest control values and 52 week control values. No organ weights of spleen, uterus, ovaries, heart, lungs, pituitary and prostate from animals necropsied at the end of the study were reported. Mean data were given for males and females together, and not separately for both sexes. In spite of these deviations from OECD guidelines, the study is considered acceptable for the overall toxicological evaluation.

Conclusions

Following oral administration of MK-0244 (emamectin benzoate) to dogs for 52 weeks, clinical signs of neurotoxicity and histological changes in central and peripheral nervous system and muscle fibre were observed at and above 0.5 mg/kg bw/day, together with decreased neutrophil number in blood. Based on these (neuro)toxic effects, the NOAEL in this study is 0.25 mg/kg bw/day.

3.12.2 Chronic toxicity and carcinogenicity

3.12.2.1 STUDY 1 - dietary carcinogenicity/toxicity study in rats

Study reference: B.6.5.1, STUDY 1

Characteristics

Type of study	:	dietary carcinogenicity/toxicity study in rats	Exposure	:	Repeated by diet, 104 weeks
Year of execution	:	1991-1993	Doses[b]	:	0, 0, 0.25, 1.0, and 5.0[c]/ 2.5[d] mg/kg bw/day
Test substance	:	MK-0244 (L-656,748-052S, technical; purity 95.9% at initiation, 97.4 to 98.6% weeks 10, 41, 60, 82 and 105)[a]	Vehicle	:	
Route	:	oral	GLP statement	:	yes
Species	:	Rat, Sprague-Dawley Crl:CD(SD)BR	Guideline	:	-
Group size	:	Test substance: 75/sex/dose Controls: 130/sex	Acceptability NOAEL	: :	acceptable 0.25 mg/kg bw/day

a: the stability of the test compound was not reported (but was determined to be satisfactory in this study by the notifier).

b: the dose of emamectin benzoate hydrate salt were calculated as base compound by using a factor of 1.15 (based on the stoichiometry of water in the MK-0244 crystal structure) (note of the notifier)

c: f: weeks 1-9 and m: weeks 1-5

d: f: weeks 10-104 and m: weeks 6-104

Study design

Rats (75/sex/dose) were given 0.25, 1.0 or 5.0 mg/kg bw/day MK-0244 for 104 weeks in their diet (doses based on 3- and 13-weeks range-finding studies with rats, see B.6.3.1. study 1 and B.6.3.3. study 1). A control group (130/sex) was included. Due to (unacceptable) weight loss and tremors in males at 5.0 mg/kg bw/day in another study of MK-0244 in week 9 (14 week neurotoxicity study in rats, see B.6.7.2 study 4), the dose of 5.0 mg/kg bw/day was reduced to 2.5 mg/kg bw/day for males starting week 6. For female rats in the 5.0 mg/kg bw/day group the dose was reduced to 2.5 mg/kg bw/day in week 10 (because of observed tremors in females at 5.0 mg/kg bw/day in week 11 of abovementioned rat neurotoxicity study (see B.6.7.2 study 4). The study performance was comparable to OECD guideline 453.

Results

Table 3.12.2.1-1 Results from a 105 week oral toxicity study in rats

Dose (mg/kg bw/day)	**0**		**0.25**		**1.0**		**5.0/ 2.5**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No treatment-related increase								
Clinical signs -unkempt -urine staining -lethargic -footsore hind	 23% 7% 29%	 9% 29%					 39% 16% 64%	 17% 40%	
Body weight gain (g)	533	361	573	329	540*	371*	430*	441*	
Food consumption	No toxicologically relevant effects								
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology (week 105) - segmented neutrophils (cells/mm^3) -eosinophils (cells/mm^3)	 4518	 39	 4822	 32	 4632	 35	 6555	 306	
Clinical Chemistry (week 105) -ALT (u/L) -CK (u/L) -potassium (meq/L) -phosporus (meq/L) -triglycerides, week 79 (mg/dL)	 449 4.9 308	 50 439 4.4 6.5 168	 486 5.2 176	 52 302 5.0 6.8 194	 319 5.4 167	 47 355 5.3 6.9 383	 266 5.8 152	 92 547 6.0 7.4 954	 m, f f m, f
Urinalysis	No toxicologically relevant effects								
Organ weights -kidney (% bw) -liver (% bw) -testes (% bw)	 0.71 2.36 0.48		 0.77 2.66 0.45		 0.90 3.00 0.43		 0.93 3.54 0.58		 m m
Pathology									
macroscopy -foot sores (plantar granulomas) -tail nodule	 29% 13%		 12%		 29%		 64% 27%		
microscopy -brain, neuron vacuolar degeneration	 0	 0	 0	 0	 0	 0	 27/28	 12/23	

-spinal cord, neuron vacuolar degeneration -kidney chronic nephritis -urinary bladder, chronic proliverative cystes	0 7%	0 53%	0 7%	0 63%	0 7%	0 63%	15/28 17%	1/23 80%	

dr = dose related; * $p \leq 0.05$

In contrast to the 13-week and 52-week studies with rats, no clinical signs of neurotoxicity were observed in this study. Increased body weight gain in females of the mid (10-20%) and high (10-28%) dose group was observed after the first weeks of the study until termination. In high dosed males loss of body weight was observed from week 46 of the study, resulting in a statistically significantly lower terminal average body weight than control males. An increased number of segmented neutrophils in high dose males in weeks 79 and 105 were observed. At all dose levels, triglyceride levels were decreased in males; however, this was likely due to high control values, which were partly higher than historical control values. In females, a dose-related increase in triglyceride levels was observed, which is considered toxicologically relevant at and above 1.0 mg/kg bw/day. These effects on blood triglyceride levels were most prominent at weeks 52 and 79, but also apparent at week 105. At the mid and high dose, males had increased relative liver and kidney weights, which were not accompanied by histopathological changes.

Terminal microscopic examination showed vacuolar degeneration in neurons of brain and spinal cord of high dose animals. These observations were most prominent in males. These changes were also observed in early death high dose animals. In high dose females a higher incidence of chronic nephritis was observed. In high dose males more chronic proliferative cystes were observed in the urinary bladder. In early death animals higher incidences of prostate chronic inflammation (high dose males), heart chronic focal myocarditis (high dose females), Harderian gland chronic inflammation (mid and high dose females) and skeletal muscle atrophy (high dose females) were observed. There was no treatment-related increase in tumours.

Acceptability

The study is considered acceptable.

Conclusions

In high dose animals several parameters were changed, the most prominent effect being vacuolar degeneration of neurons in brain and spinal cord and effects on bodyweight gain. Based on effects observed on female blood triglyceride levels and on male relative weights of kidney and liver at and above 1.0 mg/kg bw/day, the NOAEL in this study is 0.25 mg/kg bw/day. In this study with rats, no substance-related increase in tumours was observed.

3.12.2.2 STUDY 2 - Carcinogenicity study in mice

Study reference: B.6.5.1, STUDY 2

Characteristics

Type of study	:	Carcinogenicity study in mice	Exposure	:	Repeated by diet, 79 weeks
Year of execution	:	1991-1992	Doses[a]	:	m: 0, 0, 0.5, 2.5 and 12.5/7.5/5 mg/kg bw/day f: 0, 0, 0.5, 2.5 and 12.5/7.5 mg/kg bw/day
Test substance	:	MK-0244 (L-656,748-052S (Lot #2); purity 97.%)	Vehicle	:	-
Route	:	Oral	GLP statement	:	Yes, with exceptions
Species	:	Mouse, Crl:CD-1	Guideline	:	-

Group size	:	50/sex/group	Acceptability	:	acceptable
			NOAEL	:	2.5 mg/kg bw/day

a: since the compound was provided as benzoate salt and to account for the stoichiometry of water in the MK-0244 crystal structure doses of the compound were calculated as base compound by using a factor of 1.15 (except week 1 in which 1.16 was used)

Study design

The doses for this study were based upon the results of a previously conducted 13-week mice dietary toxicity study.

Mice (50/sex/dose) were given 0.5, 2.5 or 12.5 mg/kg bw/day MK-0244 for 79 weeks in their diet; two control groups were included. An additional 15/sex/dose (one control group) were added to the study for haematological examination after only 1 year. Due to an increased incidence in mortality, decreased body weights and tremors, the high dose for males was reduced to 7.5 mg/kg bw/day during week 9, and further reduced to 5.0 mg/kg bw/day from week 31 until termination (due to continued decreased weight gain/ body weight loss). For high dose female mice the dose was reduced to 7.5 mg/kg bw/day in week 48 (because of decreased weight gain). The study was initially scheduled for a duration of 93 weeks, but due to increased mortality at the high dose level, the study was terminated in week 79. The study performance was comparable to OECD guideline 451.

Results

Table 3.12.2.2-1 Results from a 79 week oral carcinogenicity study with mice

Dose (mg/kg bw/day)	**0**		**0.5**		**2.5**		**12.5/7.5/5.0**	**12.5/7.5**	**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
-Mortality (weeks 3-11) -total -Trend p-value	34%	25%	21%	22%	21% 0.188	26% 0.422	7 68% <0.001	60% <0.001	
Clinical signs -tremor -vocalization -fine fasciculating tremor of forequarter/forelimbs -skin lesions (cracking, exudate formation, hair loss, scabbing)	49%	6%	48%	12%	46%	18%	3/50 5/50 35/50 84%	5/50 45/50 74%	f
Mean body weight gain (g) Final body weight (g)	15.8 45.3	15.8 37.7	16.9 44.2	17.5 37.8	16.7 43.2	15.5 36.8	5.1* 33.6 (-26%)	7.6* 28.5 (-24%)	
Food consumption	No toxicologically relevant effect								
Water consumption	Not determined								
Ophthalmoscopy -blepharitis and corneal scarring							11/50	9/50	
Haematology (week 79) -segm. neutrophils (%) -lymphocytes (%) -ery's (million/mm^3) -Hb (g/100 mL) -MCV (cubic microns)	 7.8 12.3 45.4 35.3 6.8 3797	18 78	 8.4 13.5 45.2 35.7 5.9 3950	16 81	 8.0 13.0 44.2 36.4 7.0 4048	22 72	 7.1 11.2 47.2 33.5 3.8 2264	31 65	

-MCHC (g/dL) -leucocytes (1000/mm^3) -lymphocytes (cells/mm^3)									
Clinical Chemistry	Not performed								
Urinalysis	Not performed								
Organ weights -kidneys (% bw) -liver (% bw) -adrenals (% bw)	 0.012	 1.38 5.40 0.025	 0.012	 1.42 5.36 0.026	 0.015	 1.39 5.44 0.025	 0.016	 1.81 6.10 0.034	
Pathology									
- *macroscopy* -dermatitis -spleen enlargement -lymph node enlargement	 16 9 9	 2 4 4	 11 9 8	 2 6 3	 15 13 13	 4 7 5	 35 24 27	 36 24 23	
- *microscopy* -degeneration sciatic nerve (early death) -dermatitis -spleen, extramedullary haematopoiesis -lymph node, lymphoid hyperplasia -bone marrow, myeloid hyperplasia	 22/66 17/66 9/66 17/66	 5/75 24/75 11/75 21/75					 2 15/16 13/16 9/16 13/16	 12/20 9/20 7/20 17/20	

* $p < 0.0001$

Four high dose males were found dead during treatment weeks 3-8 (12.5 mg/kg bw/day). Three additional high dose males were found dead during treatment weeks 9-11 (7.5 mg/kg bw/day). Prior to death some mice showed tremors and vocalization (see below). Treatment-related skin lesions were seen in two of these mice, and consisted of slight degeneration of the sciatic nerve (vacuolation). The incidence of mortality from weeks 12 to 51 in high dose animals was similar to controls. After this time, the incidence of mortality increased in high dose animals, partly due to systemic infection occurring as a consequence of skin lesions (see below). Total mortality in the high dose group was 68% and 60% for males and females, respectively, compared to 34% and 25% for control males and females, respectively.

In high dose males, tremors and vocalization were observed between weeks 5 to 16. In high dose females vocalization was observed between weeks 16 to 34. From week 14 on, a fine fasciculating tremor of the forequarter/forelimbs (and rarely the hindquarter/hindlimbs) was observed in the high-dose group when the mice were suspended by their tails during handling. Occasionally sharp myoclonic movements were observed as long as the animals were held by the tail. After week 18, the incidence of these signs increased gradually and affected a large number of mice in the high-dose group. The observed skin lesions were characterized by cracking, exudate formation, hair loss and scabbing. Cervical region, ears and eyes were mostly affected. These lesions showed heavy growth of *Staphylococcus intermedies* and/or *Streptococcus Group G*. These lesions, observed most prominent in high dose mice, were observed from week 5 on and became more prominent after 6 months. This skin infection problem, which was exacerbated in high dose animals, was attributed to the poor clinical condition (e.g. markedly lower weight gain) and stress in high dose animals owing to toxicity. Therefore, it was considered to be a secondary effect of toxicity and not a direct test substance related effect. The higher incidence of infectious processess is also considered to be responsible for the proportionately higher incidence of histopathological changes in the spleen, lymph nodes and bone marrow in the high dose group (see below).

Decreased weight gain/body weight loss was observed in high dose mice during the first year. Compared to controls, the average weight gains from one year until termination of the study were approximately 50-60% and 60-70% less in high dose males and females, respectively (statistically significant).

In blood of high dose mice changed numbers of segmented neutrophils, leucocytes and lymphocytes were observed.

Relative organ weights of kidneys (f), liver (f) and adrenals (m, f) were increased in these high dose mice (probably related to lower bodyweights).

In two high dose male mice given 12.5 mg/kg bw/day degeneration of the sciatic nerve (characterized by vacuolation and the presence of small myelin balls in the nerve fibre) was seen. Infectious processes (dermatitis, abscesses and suppurative lesions in viscera) were most prevalent in mice that did not survive to termination.

In high dose mice, gross pathology showed increased incidences in dermatitis, spleen enlargement and lymph node enlargement. These effects were confirmed by microscopic examination (dermatitis, spleen extramedullary haematopoiesis, lymph node lymphoid hyperplasia). These changes in high dose animals were observed at terminal necropsy as well as in early deaths.

There was no treatment-related increased incidence in tumours observed.

Acceptability

Exposure scenarios of high dose males and females are different: high dose males exposure exists of three periods with decreasing doses, ending with 5.0 mg/kg bw/day; high dose female exposure exists of two periods, ending with 7.5 mg/kg bw/day.

Room temperature, humidity and air changes were not reported. Several deviations from GLP were reported in the GLP statement.

In spite of abovementioned deviations from OECD 451, the study is considered acceptable as carcinogenicity study.

Conclusions

Based on increased mortality, marked decreased weight gain, clinical signs of neurotoxicity, increased incidence of skin lesions, changes in haematological parameters and increased relative organ weights observed in high dose mice, the NOAEL in this study is 2.5 mg/kg bw/day. No treatment-related increase in tumour incidence was observed in mice.

3.12.3 Semi-chronic neurotoxicity

3.12.3.1 STUDY 1 - Sub-acute neurotoxicity

Study reference: B.6.7.2, STUDY 1

Characteristics

Type of study	:	Sub-acute neurotoxicity	Exposure	:	16 days
Year of execution	:	1990-1992	Doses	:	0, 0.05, 0.10, 0.30, and 0.90 mg/kg bw per day*
Test substance	:	MK-0243: L-656,748-038W002 Purity: 96.9% [a]	Vehicle	:	-
Route	:	Oral, via diet	GLP statement	:	Yes
Species	:	Mouse Crl:CF-1 Br strain	Guideline	:	-
Group size	:	10 /sex/dose	Acceptability	:	Acceptable
			NOAEL	:	0.1 mg/kg bw per day*

a L-656,748-038W002: 96.9% epi-methylamino avermectin (B1a + B1b') and 92.1% epi-amino avermectin (B1a)

* no factor is mentioned in the study report;Other studies with this batch used a conversion factor of 1.14, so it may be assumed that this was used for this study.

Study design

Groups of 10 mice/sex were orally dosed via their feed with L-656,748-038W at a level of 0, 0.05, 0.10, 0.30 or 0.90 mg/kg bw per day. This specific mouse strain was chosen since it has been demonstrated to be extremely sensitive to the toxicity induced by this class of compounds.

In this study, animals were examined for mortality, clinical signs, body weight and food consumption. All animals were examined for gross lesions. For animals of the control and high dose group and also for animals sacrificed in moribund condition, brain weight was determined and histopathology was performed on brain, spinal cord and sciatic nerves.

Results

No clinical signs were observed in either of the two low dose groups. Of the 20 mice in each of the upper two dose groups (0.3 and 0.9 mg/kg), four to six mice developed dose-related clinical signs including tremors and/or decreased activity and four in each group were killed in moribund condition.

In the high dose group the first signs of neurotoxicity were observed already 24 - 48 hours after dosing. In the 0.3 mg/kg group signs of toxicity were first observed on day 3 of dosing.

In male mice, mean weight gain in the 0.3 mg/kg group was depressed compared to control, primarily due to one animal that lost 9.8 g and showed tremors and other signs of toxicity. Another male in this group lost 3 g but did not show any clinical signs of toxicity. In the low dose groups nor in the high dose group, effects on body weight were observed.

None of the animals, including those killed in moribund condition, showed gross lesions. Further, no histopathological changes were observed in the high dosed animals and in the animals killed in moribund condition.

Acceptability

The study is acceptable, although not performed according to OECD 424 (no functional observational battery and motor/locomotor activity measurements were performed).

Conclusions

Clinical signs of neurotoxicity were observed at 0.3 and 0.9 mg/kg bw per day, and four mice in each of these dose groups were killed in moribund condition. No clinical signs of neurotoxicity or reduced body weight gain were observed in CF-1 mice dosed up to and including 0.1 mg/kg bw per day.

3.12.3.2 STUDY 2 - Sub-acute neurotoxicity

Study reference: B.6.7.2, STUDY 2

Characteristics

Type of study	:	Sub-acute neurotoxicity	Exposure	:	2 weeks
Year of execution	:	1989-1992	Doses	:	0, 0.2, 0.6, 1.2, and 2.0 mg/kg bw per day*
Test substance	:	MK-0243: L-656,748-010V003 Hydrochloride salt	Vehicle	:	-

		Purity: 96.1% [a]			
Route	:	Oral, via diet	GLP statement	:	No
Species	:	Mouse Crl:CD-1(ICR) Br strain	Guideline	:	-
Group size	:	10 /sex/dose	Acceptability	:	Acceptable
			NOAEL	:	> 2 mg/kg bw per day

a L-656,748-010V003: 96.1% B1a + B1b (with 0.76% propylgallate added as an oxidant)
* dose levels are expressed as base compound (factor 1.04).

Study design

Groups of 10 mice/sex were orally dosed via their feed with L-656,748-010V at a level of 0, 0.2, 0.6, 1.2, and 2.0 mg/kg bw per day.

In this study, animals were examined for mortality, clinical signs, body weight and food consumption. All animals were examined for gross lesions. For animals of the control and high dose group, brain weight was determined and histopathology was performed on brain, spinal cord and sciatic nerves.

Results

No deaths or test substance-related clinical signs occurred at any dose level. Body weight gain was increased in males in the 0.6 and 1.2 mg/kg groups, but not in males at higher or lower dose levels or in females at any dose level. The alteration in body weight was considered test substance-related by the study authors; however, the mechanism of this change is not known. Food consumption was unaffected by test substance administration at all dose levels. No test substance-related gross or microscopic lesions were apparent in any of the tissues examined from the high dose group. However, one high dose male had a slight, cellular infiltrate in the meninges of the brain. This probably indicates an asymptomic viral infection, occassionally seen in control mice in the laboratory in question.

Acceptability

The study is acceptable. Study is not performed according to OECD 424, since no functional observational battery and motor/locomotor activity measurements were performed.

Conclusions

No clinical signs of neurotoxicity were observed in CD-1 mice dosed up to and including 2.0 mg/kg bw per day, the highest dose tested.

3.12.3.3 STUDY 3 - Sub-acute neurotoxicity

Study reference: B.6.7.2, STUDY 3

Characteristics

Type of study	:	Sub-acute neurotoxicity	Exposure	:	5 weeks
Year of execution	:	1989-1992	Doses	:	0, 0.5, and 1.5 mg/kg bw per day*
Test substance	:	MK-0243: L-656,748-010V003 Hydrochloride salt Purity: 96.9% [a]	Vehicle	:	Deionised water
Route	:	Oral, gavage	GLP statement	:	No
Species	:	Beagle dog	Guideline	:	-
Group size	:	1-3/sex/dose	Acceptability	:	Acceptable
			NOAEL	:	0.5 mg/kg bw per day

a L-656,748-010V003: purity 96.6% with 0.76% propylgallate added as an oxidant

* dose levels are expressed as base compound (factor 1.04).

Study design

Three groups of young adult beagle dogs were treated orally, by gavage, with L 656,748-010V dissolved in water for up to 33 days at dose levels of 0 (1 animal/sex), 0.5 (2 animals/sex) or 1.5 mg/kg bw per day base compound (3 animals/sex). The control male and one dog of each sex from both treated groups were killed after 7 days of test substance administration. One male and one female dog from the high dose group (1.5 mg/kg bw) were killed after 22 days of test substance administration. The remaining animals (the control female and 1 per sex per dose group) were killed at termination of the 33 day dosing period. The animals were observed daily for mortality and clinical signs; food consumption and body weights were recorded weekly. All animals were subjected to a necropsy limited to brain, spinal cord and sciatic nerves and subjected to perfusion fixation. Sections of brain, 3 levels of spinal cord and proximal and distal sciatic nerves were processed to haematoxylin and eosin stained sections and examined by light microscopy. Sections of mid-brain and medulla were also stained immunohistochemically to detect phosphorylated epitopes on neurofilaments, and sections of stained mid-brain and sciatic nerves from selected animals were examined by electron microscopy.

Results

One male in the 1.5 mg/kg group was killed in a moribund condition in week 4. The animal had lost weight and showed ataxia, mydriasis, tremors, and salivation. Tremors, mydriasis and ptyalism also occurred from week 2 in the other animals of the 1.5 mg/kg bw group, but not at 0.5 mg/kg bw. These signs occurred about 4-6 h post-treatment. Test substance-related decreases in food consumption and body weight occurred in the 1.5 mg/kg group only. No gross lesions were evident at necropsy. Test substance-related histomorphological alterations occurred only in animals in the 1.5 mg/kg group after at least 13 days (found in animals of the second interim sacrifice and in the moribund and terminal sacrified animals). The effects comprised neuronal and white matter degeneration in the brain and spinal cord and peripheral nerve degeneration. Very slight to moderate neuronal degeneration was characterised by swollen neurons with eosinophilic cytoplasm and frequently, acentric nuclei. The perikaryon of degenerating neurons stained positive for phosphorylated epitopes on neurofilaments (normally only unphosphorylated neurofilaments are found in the perikaryon). Slight to marked white matter degeneration was characterised by multiple vacuolation, occasionally containing eccentric axons, or myelin and/or cell debris.

Very slight to slight peripheral nerve degeneration had similar characteristics. Other than a few slightly swollen neurons, electron microscopy revealed no further neuronal alterations.

Acceptability

The study is acceptable. Study is not performed according to OECD 424, since no functional observational battery and motor/locomotor activity measurements were performed.

Conclusions

Occurrence of weight loss, reduced food consumption, clinical signs of neurotoxicity and histomorphological alterations in central and peripheral nerve tissues were observed in dogs dosed at 1.5 mg/kg bw per day. No effects were observed in dogs dosed at 0.5 mg/kg bw per day.

3.12.3.4 STUDY 4 - Semi-chronic neurotoxicity

Study reference: B.6.7.2, STUDY 4

Characteristics

Type of study	:	Semi-chronic neurotoxicity	Exposure	:	14 weeks
Year of execution	:	1991-1992	Doses	:	0, 0.25, 1.0 and 5.0 mg/kg bw per day*
Test substance	:	MK-0244: L-656,748-052S002 Purity: 95.9%	Vehicle	:	-
Route	:	Oral, via diet	GLP statement	:	yes
Species	:	Rat Crl:CD(SD) Br strain	Guideline	:	-
Group size	:	10/sex/dose	Acceptability	:	Acceptable
			NOAEL	:	1 mg/kg bw per day*

* all dose levels are expressed as base compound (factor 1.15).

Study design

Groups of 10 m/f young adult rats were administered L-656,748-052S orally for 13 weeks, in the diet, at variable concentrations calculated to provide target dose levels of 0, 0.25, 1.0 or 5.0 mg/kg bw per day. A second group of 10 female rats was used as an ancillary control group. Mortality and clinical observations were recorded daily, and body weights and food consumption were measured weekly. The animals were subjected to a battery of neurological function tests and observations (FOB) and assessment of locomotor activity (LMA) prior to dosing, and during weeks 5, 9 and 13. The FOB included home cage/hand held and open field observations, response observations in a testing arena, performance measures (including grip strength, foot splay on landing, rectal temperature and tail flick latency). After at least 13 weeks of test substance administration, whole body perfusion was performed on all main study animals. Central and peripheral nervous tissues, viz. brain, spinal cord, cervical and lumbar dorsal root ganglia, cervical and lumbar dorsal and ventral root nerves, Gasserian ganglia, sciatic nerve and branches and optic nerve were preserved. Skeletal muscle was also preserved. Tissues from 6 animals/sex from the control, low and high dose groups and all animals of the mid dose group were prepared for histological evaluation as paraffin-embedded sections and/or plastic-embedded thin sections, as appropriate. These tissues, except plastic embedded tissues from the low dose group, together with all gross lesions from all animals, were examined microscopically. Neurobehavioural data were statistically analysed by trend testing, both by individual test and by functional category, viz. muscle tone and equilibrium, sensorimotor responses, central nervous system and autonomic nervous system.

Results

The results are summarized in table 3.12.3.4-1.

Table 3.12.3.4-1: results from a 14-week dietary neurotoxicity study in the rat

	Dose level (mg/kg bw per day)								**dr**
	control		**0.25**		**1.0**		**5.0**		
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortality								
Clin signs (1)									
alopecia		1	1	1	1				
scabs			2	1					
abdominal swelling					1*				
salivation							6		
body tremors							8		
rough, soiled coat							6	2	
Body weight							d		
Food cons							d		
Neurotox evaluation: **									
- Spontaneous activity (2)									
home cage posture							a [ss]		
convulsions							a [ss]	a	

	Dose level (mg/kg bw per day)								dr
	control		0.25		1.0		5.0		
	m	f	m	f	m	f	m	f	
fur appearance							a ss		
rearing							a ss		
- Autonomic trend (3)									
salivation							a ss		
- Muscle tone equilibrium-trend (4)									
gait							a ss		
gait score							a ss		
mobility score							a ss		
forelimb grip strength							a ss	a ss	
hindlimb grip strength							a ss		
righting reflex							a ss	a ss	
- Motor & sensory affective trend (5)	No effects observed								
- Motor activity (6)	No effects observed								
Microscopic examination:									
- brain:									
neuron, cytopl. vacuolisation					0/10	0/10	6/6	6/6	
- spinal cord									
neuron, cytopl. vacuolisation					0/10	0/10	6/6	6/6	
white matter degeneration	1/6	1/6			1/10	0/10	6/6	4/6	
- sciatic nerve									
degeneration	1/6				1/10		6/6	1/6	
- optic nerve			No effects observed						
- skeletal muscle ***									
atrophy							3/7		
chron. focal myositis			1/6					1/8	

d = decreased; i = increased; a = indicates an adverse effect on parameter; ss = statistically significant

* A necropsy an undescended testicle was found to correspond with this swelling (which is considered a commonly observed finding in rats).

** Summary of functional Observational Battery (FOB) and Motor Activity (MA) results; only the parameters affected are mentioned.

*** A higher number of total animals examined is presented here, because there were also some additional animals with gross lesions.

(1) Number of animals involved;

(2) Scored parameters: home cage posture, convulsions, vocalisation, removal difficulty, handling difficulty, fur appearance, arousal, rearing.

(3) Scored parameters: lacrimation, salivation, piloerection, defaecation, urination, pupil response.

(4) Scored parameters: gait, gait score, mobility score, forelimb grip strength, hindlimb grip strength, landing (hindlimb) foot splay, righting reflex.

(5) Scored parameters: handling palpebral closure, approach response, click response, touch response, tail flick.

(6) Scored parameters: horizontal activity.

There was no mortality during the study and test substance-related clinical signs (salivation,

body tremors and rough, soiled coat) were confined to the high dose group, especially in males. The signs were first seen in week 7 in males and in week 11 in females and lasted for the remainder of the study. Other clinical signs observed in the lower dose groups were not considered related to treatment. Overall reduction of weight gain in males of the high dose group was about 25% compared with controls. The effects on weight gain were first noted in week 8, when clinical signs started to be seen. Total mean food consumption was not affected, but in males at the high dose only, lower consumption was observed in the last two weeks of the study.

Test substance-related, statistically significant effects in the neuro-behavioural tests were confined to both sexes in the 5.0 mg/kg bw per day group at weeks 9 and 13 only, with males more affected than females. Specific effects in males were mild convulsions (i.e. tremors), salivation, rear-limb splay, impaired gait and righting reflex, hind-limb paresis, soiled fur and reduced rearing activity and grip strength (30 - 50%). Grip

strength was reduced by 20 - 30% in females in weeks 9 and 13, and one female showed mild tremors in week 13. No test substance-related effects on motor activity were observed at any dose level.

Histopathological effects occurred only in the 5.0 mg/kg bw per day group. Very slight to slight cytoplasmic vacuolation of large neurons occurred in the brain and spinal cord of all examined animals (m/f) in this group. The affected neurons were primarily in the lateral aspects of the brainstem at the level of the diencephalon, pons and trapezoid body, and in the mid-lateral to ventral aspects of the spinal cord grey matter. These effects did not occur in any animal in the 1.0 mg/kg bw per day group. Very slight to moderate degeneration of white matter in the spinal cord, characterised by damage to myelin sheaths and axons and accumulation of myelin debris and macrophages, was also apparent in all examined males and most females of the high dose group. Very slight to moderate treatment-related degeneration of myelinated fibres in the sciatic nerve occurred in all males, but only in one female of the high dose group.

Very slight to marked atrophy was seen in the skeletal muscle sections of several high dose male rats. This change was not present in the female rats of this group or in rats of either sex from the other groups examined. No microscopic alterations occurred in the optic nerve at any dose level.

Acceptability

The study was performed in accordance with OECD guideline 424, with the following deviations: no ophthalmological examiniations have been performed. Despite this deviation, the study is considered acceptable.

Conclusions

Dietary administration of L-656,748-052S at a dose level of 5 mg/kg per day produced both clinical and histopathological evidence of neurotoxicity in both male and female rats (mild tremors, effects observed in the FOB, decreases in bodyweight gain in male rats, neuronal vacuolation in the brain and spinal cord, degeneration of nerve fibres in the spinal cord and sciatic nerve, and skeletal muscle atrophy in some high dose males) with, in general, more pronounced effects observed in males. No clinical or histopathological evidence of neurotoxicity was observed at lower dose levels tested in this study (0.25 and 1 mg/kg per day). The NOAEL for neurotoxicity was 1 mg/kg bw per day.

3.12.4 Human data

No data available.

3.12.5 Other data

No data available.

3.13 Aspiration hazard

No data available.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Study reference: Dietschy, A. (1999), STUDY IIA, 7.7/01

Reference/notifier	:	Dietschy, A. (1999)	GLP statement	:	yes
Type of study	:	ready biodegradability	Guideline	:	OECD 301 F; 92/69/EEC, L383 A, C.4-D
Year of execution	:	1999	Acceptability	:	acceptable
Test substance	:	MK 244 (emamectin benzoate), batch nr. FL 971780, chemical purity 96.1 %, white powder			

Substance	Water type	T [°C]	pH	Duration [d]	Transformation at end [%]	Classification
emamectin benzoate	activated sludge	21	7.3 – 8.2	28	3	not readily biodegradable

Materials and methods

Test substance was added to a mineral salts medium inoculated with activated sludge, suspended solids concentration 30 mg/L, test concentration 100 mg/L. Duplicate vessels for:

- test substance (medium + inoculum + emamectin benzoate),
- Inoculum control (medium + inoculum),
- positive (procedure) control (inoculum + medium + sodium benzoate),
- abiotic control (medium without inoculum + emamectin benzoate + sterilising agent $HgCl_2$
- toxicity control (inoculum + medium + emamectin benzoate + sodium benzoate).

Incubation for 28 days in the dark. BOD was measured continuously.

Calculations. Biodegradation calculated as BOD/Theoretical Oxygen Demand.

Results

Oxygen consumption in blank control 12 – 38 mg/L after 28 days. Biodegradation of emamectin benzoate was 0 % after 28 days. Biodegradation of sodium benzoate was 79 % at day 14 and 80 % after 28 days, biodegradation in toxicity control was 60 and 63 % after 14 and 28 days. Abiotic control had 0 % degradation after 28 days.

4.1.2 BOD_5/COD

No data available.

4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

4.1.4.1 Study 1 - hydrolysis

Study reference: A.C. Chukwudebe (1992), STUDY IIA, 7.5.1/01

Detailed study summary and results:

Reference/notifier	:	A.C. Chukwudebe (1992)	GLP statement	:	yes
Type of study	:	hydrolysis	Guideline	:	OECD 111; US-EPA Subdivision N, 540/09-82-021, section 161-1 BBA 55, I and II
Year of execution	:	1989	Acceptability	:	Acceptable
Test substance	:	[3,7,11,13,23-^{14}C]4"-deoxy-4"-epimethylamino avermectin B_{1a} benzoate; batch nr. L-683,825-003E001; radiochemical purity 93.6%			

Substance	Buffer type	T [°C]	pH	Duration [w]	Transformation at end [%]	DT_{50} hydrolysis [weeks]
^{14}C-MAB_{1a}	potassium hydrogen phtalate	25	5.2	6	0	Stable
	phosphate	25	6.2	6	0	Stable
	phosphate	25	7.2	6	0	Stable
	sodium tetraborate decahydrate	25	8	6	0	Stable
	sodium tetraborate decahydrate	25	9	6	26.5	19.5[1]

[1] extrapolated beyond study duration

Methods

Duplicate samples of 8.9 mg ^{14}C-MAB_{1a}/L were prepared in 0.01 M sterile buffers at pH 5, 6, 7, 8 and 9 at 25°C for 6 weeks, each. Samples were withdrawn at 0, 1, 2, 4, 5 and 6 weeks. Temperature, pH and sterility confirmed. Control samples were prepared and stored in the freezer until use.

Chemical analysis. 0.35 ml of methanol was added to 50 µL aliquot of the sample and mixed with 100 µL of buffer solution. A histogram of the radioactivity of the effluent from the RP-HPLC of 50 µL of the buffer-methanol mixture was obtained and a 50 µL aliquot was taken for radioactivity assay by LSC.

Calculations. Hydrolysis rate was determined by linear regression analysis of the hydrolysis data.

Results

The average percentage analysed was calculated by comparison of the amount of radioactivity injected with the theoretical value calculated from the control data. Average percent of radioactivity remaining in the solution (rest is adsorbed to the walls of the container) was 74.0 % for pH 9 to 90.8 % for pH 6. Average percentage analysed ranged from 88.3 % to 101%. No correlation between time and amount of radioactivity. Therefore, losses could not be accounted for by the formation of a volatile hydrolysis product. Average HPLC recovery 98.1 to 99.0 % of AR for the different pHs. Average percentage of the total recovered radioactivity found at the retention time of MAB1a was 75 % at pH 9 and between 90.9 and 93.5% for pHs 5.2 to 8. After 6 weeks, only MAB1a in pH 9 buffer showed significant differences between 0 time and 6-week samples.

A $DT_{50,hydrolysis}$ of 19.5 weeks was determined for pH 9. Two products, A and B, with relative retention times to MAB1a of approximately 0.21 and 1.16 respectively, were observed to form during the hydrolysis of MAB1a in pH 9 buffer. Products were 9.1 and 9.9 % of radioactivity for A and B, respectively. At four weeks the products would be represented at 6.3 and 6.9 % of radioactivity. Since neither product A nor B represented 10% or more of the total radioactivity after 30 days in pH 9 buffer, identification was not pursued. The mass at the retention time of MAB1a was isolated from the pH 9 and was confirmed to be the test compound by NMR and MS.

4.1.4.2 Study 2 - Degradation in water-sediment systems

Study reference: Hurt, AD, Grosjean, J, Mason, G (2006), STUDY IIA, 7.8.3/001 (IIA, 7.2.1.3.2/001)

Detailed study summary and results:

Reference/notifier	:	Hurt, AD, Grosjean, J, Mason, G (2006)	GLP statement	:	Yes
Type of study	:	Aerobic degradation in water-sediment systems	Guideline	:	OECD Guideline 308
Year of execution	:	2006	Acceptability	:	acceptable
Test substance	:	[23 ^{14}C]-emamectin benzoate B_{1a}, batch CDC-XV-21-1, radiochemical purity > 93.4%, appearance unknown			

Substance	Soil type	Ratio sediment water [g dwt/mL]	T [°C]	pH	OM [%]	Duration [d]	Degradation at end [%]	DT_{50} water [d][1]	DT_{50} sediment [d]	DT_{50} system [d]
[23-^{14}C]- emamectin benzoate B_{1a}	silt loam	1:4	20±2	6.99-7.98	7.4	120	20.9	10.61	n.d.	> 120
	sand			4.96-9.80	1.5	120	11.9	8.84	n.d.	>120

n.d.: not determined
1: $DT_{50,water}$ mainly caused by sorption to sediment. $DT_{50,water}$ is based on dissipation.

Methods:

Water/sediment systems. Two types of aerobic water/sediment systems were set up and dosed with [23-^{14}C]-emamectin benzoate B_{1a} to determine degradation kinetics of the test substance and to determine the presence of (relevant) metabolites.

Silt loam. Calwich Abby lake sediment from Calwich (Derbyshire, United Kingdom): 40 g dwt sediment (98.79 g wwt); CEC 165 mmol/kg. Microbial biomass sediment 298.8 mg C/kg at start of test.

Sand. Haut Languedoc lake sediment from Haut Languedoc (France): 40 g dwt sediment (51.49 g wwt); CEC 47 mmol/kg. Microbial biomass sediment 32.9 mg C/kg at start of test.

Sediment wet sieved, 2 mm, corresponding water passed through 0.212 mm. Incubation flasks filled with 98.79 and 51.49 g wet sediment (corresponding to 40 g dry sediment) for silt loam and sand, respectively, and natural waters were added to a total weight of 200 g (sediment/water volume ratio ± 1:4). Equilibration for 20 days at ± 20 °C in the dark.

Application, incubation and sampling. [23-^{14}C]-emamectin benzoate B_{1a} was applied as a solution in ethanol (final < 0.1 % v/v) into the water phase. Application rate was equivalent to five times the direct over spray rate for the highest annual application of 1250 µg/L incorporated into a 30 cm deep water body (equivalent to 103 µg applied to each vessel). Incubation at 20 ± 2 °C in the dark in glass vessels. Water and sediment were sampled in duplicate on t = 0 and after 14, 21, 28, 60, 90 and 120 days. A second application (after 120 days) was sampled 2, 6 and 16 hours after treatment to enable a more accurate determination of the dissipation rate from the water phase. In this case, only the water phase was analysed. Redox potential, pH and oxygen content were recorded on day 5 (6), 13, 20, 27, 42, 57, 89 and 119. Volatiles trapped in NaOH (2 traps) were sampled after 28, 60, 90 and 120 days.

Chemical analysis.

Water. Radioactivity directly determined by LSC. All water samples containing > 5% of applied radioactivity were further analysed by HPLC. When necessary, aliquots were concentrated by either turbo or rotor evaporation and re-analysed by HPLC-UV.

Sediment. Extractions: Step 1. Sediment was transferred to centrifuge tube and sediment vessels were rinsed with acetonitrile: 100 mM ammonium acetate (80:20 v/v), which was added to the centrifuge tube. Centrifuge tube was filled up to a total volume of 100 mL, sealed and agitated for 30 minutes on a wrist

action shaker, followed by centrifugation at 3000 rpm and 10 °C for 10 minutes. This procedure was performed twice. Step 2: The second extraction was followed by a 10 mL acetonitrile wash of the aspiration tubing. Samples from the storage bottle were analysed by LSC (and HPLC if samples contained >5% of applied radioactivity). Step 3: An additional 100 mL of acetonitrile:acidified water (pH 3 using formic acid) (3:1 v/v) was added to the wet sediment remaining in the centrifuge tube. Single extraction was performed as described above, but the supernatant was aspirated into a separate bottle. Step 4: The remaining sediment was air-dried, grinded and combusted. Quantification was done by LSC.

Volatiles. Radioactivity in trapping solutions determined directly by LSC.

Glassware. After removal of all fractions, glassware was rinsed four times with 10 mL acetonitrile (in between the vessels were placed in an ultra sonic bath). After making up the volume to 50 mL, aliquots were analysed by LSC.

Reference compounds: Emamectin benzoate B_{1a} (batch no. GAN-XL111-57, purity 95.9% w/w) was included as reference compound to determine the purity of the test substance and for the characterisation of residues in surface waters and sediment extracts by HPLC.

Calculations. Dissipation of [23-^{14}C]-emamectin benzoate B_{1a} from the water phase is estimated by using a first order multi compartment model within the curve fitting program ModelManager. Due to the rapid dissipation of the test compound from the water phase, the initial concentration in the water phase was set to 100% of applied in the model.

Results

Silt loam: Redox potential sediment 272 - 409 mV. DO water phase 2.2 - 5.6 mg/L, pH 6.99 - 7.98. Sand: Redox potential sediment 505 - 603 mV. DO water phase 3.6 - 6.6 mg/L, pH 4.96 – 6.80.

Three (or more) metabolites were observed in the water and sediment phase: metabolite(s) 1 (HPLC retention time 1.5-2.0 min), metabolite 2 (HPLC retention time 2.2 min) and metabolite 3 (HPLC retention time 2.5 min).

Distribution of radioactivity for the [23-^{14}C]-emamectin benzoate B_{1a} incubations is given in Table 4.1.4.2-1 (first application) and Table 4.1.4.2-2 (second application) for silt loam and sand systems.

Table 4.1.4.2-1Distribution of radioactivity in silt loam (Calwich Abbey, UK) and sand (Haut Languedoc, France) system after incubation with [23-^{14}C]-emamectin benzoate B_{1a}. All values represent % of AR.

Compartment/fraction	Incubation period [d]						
	0	14	21	28	60	90	120
Silt loam, Calwich Abbey							
Water							
total radioactivity	**78.4**	23.4	14.2	14.1	11.0	7.4	4.2
Sediment							
.7 Extractable	14.8	64.3	70.7	67.8	70.4	70.1	**73.0**
Non-extractable	1.8	10.8	13.8	11.9	16.0	15.2	**20.2**
CO_2	n.a.	0.9	**1.4**	1.1	0.5	0.3	0.3
Total	95.0	99.4	100.1	94.9	97.9	93.0	97.7
emamectin benzoate B_{1a}							
water	**78.4**	18.9	9.7	7.5	6.3	3.2	0
sediment	13.8	63.7	68.4	62.2	68.6	68.7	**71.3**
total system	92.2	82.6	78.1	69.7	74.9	71.9	71.3
metabolite 1							
water	n.d.	4.6	2.7	2.7[1]	2.7	2.0	n.a.
sediment	1.0[1]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
total system	1.0	4.6	2.7	2.7	2.7	2.0	n.d.
metabolite 2							
water	n.d.	n.d.	0.9[1]	**2.7**[1]	2.1	2.0	n.a.

Compartment/fraction	Incubation period [d]						
	0	14	21	28	60	90	120
sediment	1.1[1]	0.8[1]	0.7[1]	**3.1**[1]	n.d.	n.d.	n.d.
total system	1.1	0.8	1.6	**5.8**	2.1	2.0	n.d.
metabolite 3							
water	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.
sediment	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.
total system	n.d.	n.d.	n.d.	n.d.	0.8	n.d.	n.d.
Sand (Haute Languedoc)							
Water							
total radioactivity	**92.0**	25.9	15.7	12.7	10.8	10.0	8.8
Sediment							
Extractable	7.1	74.8	81.4	**85.7**	84.9	84.5	82.4
Non-extractable	0.2	4.8	5.9	5.3	8.4	8.7	**10.7**
CO_2	n.a.	0.7	1.0	**1.3**	0.6	0.6	0.7
Total	99.2	106.1	104.0	105.0	104.6	103.8	102.6
emamectin benzoate B_{1a}							
water	**83.4**	18.1	9.9	4.1	2.6	1.8	1.0
sediment	6.7	74.8	79.6	72.1	79.7	**83.0**	77.2
total system	90.1	92.9	89.5	76.2	82.3	84.8	78.2
metabolite 1							
water	n.d.	2.7	**5.8**	**5.8**	4.5	3.7	n.d.
sediment	n.d.	n.d.	n.d.	n.d.	n.d.	0.4[1]	n.d.
total system	n.d.	2.7	**5.8**	**5.8**	4.5	3.7	n.d.
metabolite 2							
water	n.d.	0.7[1]	n.d.	2.9	**3.7**	n.d.	n.d.
sediment	n.d.	n.d.	1.2	1.4	n.d.	n.d.	n.d.
total system	n.d.	0.7	1.2	**4.3**	3.7	n.d.	n.d.
metabolite 3							
water	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
sediment	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
total system	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.a.: not analysed
n.d.: not detected
1: single measurement (compound not detected in replicate)

Only 0.1 to 0.3% of applied radioactivity was found to adhere to the wall of the test vessel.

Table 4.1.4.2-2. Dissipation of [23-^{14}C]-emamectin benzoate B_{1a} in silt loam (Calwich Abbey, UK) and sand (Haut Languedoc, France) system after incubation with [23-^{14}C]-emamectin benzoate B_{1a} (analysis of water phase only). All values represent % of AR.

Compartment/fraction	Incubation period [h]		
	2	6	16
Silt loam, Calwich Abbey			
Water			
emamectin benzoate B_{1a}	44.1*	**74.6**	63.2
Sand, Haut Languedoc			
Water			
emamectin benzoate B_{1a}	**86.1**	75.9	80.1

*: According to the authors, the low value is thought to be due to a non-homogeneous sample.

The authors calculated DT_{50} values for water and system based on emamectin benzoate B_{1a} in extracts and by setting the initial concentration in the water phase to 100% of applied in the model. The values from the second application (analysis of water phase only) were also included in the modelled data set. The DT_{50} for the total system is estimated using first order multi compartment modelling. Results are given in Table 4.1.4.2-3.

Table 4.1.4.2-3. DT_{50}-values as calculated by author.

System	Label	Compartment	DT_{50} (days)	DT_{90} (days)	r^2	n
silt loam	^{14}C	water	0.40	67.5	0.74	10
		total system	not given	not given		
sand	^{14}C	water	1.73	21.0	0.96	10
		total system	not given	not given		

According to the authors, the difference in DT_{50} between the two systems was caused by intrinsic differences in sediment characteristics. This resulted in a deeper water column for the sand system. Therefore, it took the test substance longer to reach the sediment.

Remarks

According to the authors, volatiles trapped in NaOH (2 traps) were sampled after 28, 60, 90 and 120 days. However, volatiles were also reported for day 7 and 14.

Height of water column and thickness of sediment layer were not reported, the ratio water-sediment (dw) was 4:1. The amount of sediment (40 g dwt) was below the recommended amount in OECD guideline 308 (a minimum of 50 g dwt).

It is unclear whether the water phase was directly injected into the HPLC system or that it was extracted/diluted with an organic solvent first. Water samples should be diluted with acetonitrile prior to analysis.

Observed metabolites were not identified, but metabolite 1 reached an amount of applied radioactivity of > 5% (5.8%) on two subsequent time steps in the water phase of the sand water/sediment system. The applicant is requested to identify this metabolite or show that this peak is caused by multiple metabolites.

$DT_{50,water}$ is mainly determined by sorption. $DT_{50,water}$ was recalculated without setting the concentration on t=0 to 100%. This results in the following values :

- Aerobic $DT_{50,water}$: 8.7 days (χ^2 = 18.9) for silt loam and 8.7 days (χ^2 = 22) for sand. Values represent dissipation.

Degradation half-lives for the system could not be calculated, because the test substance did not degrade in the whole system for more than 23% and 13% in the silt loam and sand system, respectively. The measured data for the whole system showed no clear decline. It can be concluded that the $DT_{50,system}$ > 120 days. A $DT_{50,sediment}$ could not be calculatated as there appears to be no degradation in the sediment.

4.1.4.3 Study 3 - Degradation in water-sediment systems

Study reference: Clark, A. (2003), STUDY IIA, 7.8.3/002 (IIA, 7.2.1.3.2/002)

Detailed study summary and results

Reference/notifier	:	Clark, A. (2003)	GLP statement	:	yes
Type of study	:	water/sediment degradation	Guideline	:	EPA Guideline 162-4
Year of execution	:	2002	Acceptability	:	partly acceptable
Test substance	:	[23-^{14}C]-emamectin benzoate B_{1a}, batch JWP-X-47,			

radiochemical purity 97.07%, chemical purity 99.7%, appearance unknown

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH [KCl]	pF	Duration [d]	DT50 [d]
[^{14}C]- emamectin benzoate B_{1a}	sandy loam	aerobic[1]	0.015	25	0.5	8.3	2.2	100	63.7
[^{14}C]- emamectin benzoate B_{1b}	sandy loam	aerobic[1]	0.015	25	0.5	8.3	2.2	100	71.6

Methods

Water/sediment systems. An aerobic water/sediment system was set up: Sand: Wonder valley (Fresno County, California, USA): 50 g dwt sediment, corresponding wwt unknown. CEC 26 mmol/kg. Microbial biomass aerobic sediment 105.11 mg C/kg at start of test and 136.32 mg C/kg at the end.

Incubation flasks filled with 50 g dwt sediment and ca. 500 mL corresponding water. Sediment depth and height of water phase unknown. Control samples containing only water were also included. Equilibration for 7 days at 25 ± 1 °C in the dark under ventilation with air (aerobic).

Application, incubation and sampling.

Experiment 1 (sediment/river water set): Test substance was applied as a solution in ethanol (31 µL) into the water phase, total amount 80.26 µg per system (0.165 mg/L overlying water).

Experiment 2 (control water set): Test substance was applied as a solution in ethanol (28 µL) into the water, total amount 72.5 µg per system (0.145 mg/L). Test bottles contained only water.

All glassware was pre-coated with unlabelled emamectin in an attempt to saturate the glass binding sites (preliminary work showed glass adherence of emamectin). According to the authors, it did not eliminate emamectin adhesion to glass, but it did reduce the binding.

Water and sediment were sampled in duplicate after 0, 3, 7, 14, 21, 30, 45, 58, 80 and 105 days. Same sampling intervals were used for weight and aerobicity measurements. Room air flow is 2-5 mL/min. Volatiles were trapped in a vial containing two polyurethane foam plugs (for organic volatile radiocarbon), followed by an empty vial and two KOH solution traps (for $^{14}CO_2$). Trap solutions were replaced ± every 6 weeks.
Microbial biomass of aerobic sediment was determined by glucose amendment according to Anderson and Domsch at start and end of experiment.

Chemical analysis.

Water. Radioactivity determined by TLC. HPLC was used as confirmatory method of quantitation.

Sediment. Aqueous fraction was decanted, the sediment subsequently nitrogen-dried and transferred to another bottle. The original bottles were rinsed with methanol (or acetonitrile:reverse osmosis water (80:20)) to recover radiocarbon bound to the glass. The sediment was extracted with 100 mL acetonitrile: reverse osmosis water (100 mM ammonium acetate) (80:20) and shaken for 15-30 minutes. Next, sediments were centrifuged for 15-30 minutes at 8000 rpm. Supernatants were transferred to a separate container and the extraction was repeated with 50 mL. Supernatants from both extractions were combined and analysed by TLC and HPLC.

Following the extraction with acetonitrile:water, the sediments from day 7 to 105 were extracted twice following the same procedure, but using 3:1 acetonitrile: acidified water (0.5 HCl, adjusted to pH 6.0). Extracts were analysed by two-dimensional TLC and HPLC (5 different HPLC methods).

Next, samples from day 30 to 105 were extracted overnight with a 0.5 N NaOH (150 mL) solution to characterise the bound radiocarbon to humin, fulvic acid and/or humic acid.

Finally, the remaining sediments were air-dried, homogenised and combusted.

Parent compound and observed metabolites in aqueous phase and extractions were isolated from the TLC plates and analysed by HPLC and LC/MS.

Volatiles. Radioactivity in KOH solutions determined directly by LSC, foam plugs were first dissolved in ± 20 mL of Scintisafe and then assayed, CO_2 confirmed by $BaCO_3$-precipitation and assays of the resulting supernatant.

Reference compounds: emamectin benzoate B_{1a} (batch no. S97-2162, purity 96.6%), emamectin benzoate B_{1a} (batch no. GAN-XLIII-57, purity 94.1%), 8,9-Z-MAB_{1a} (batch no. DAH-XXV-77, purity 87.8%), 8,9-Z-MAB_{1a} (batch no. DAH-XXVII-50, purity 89.9%), 8,9-Z-MAB_{1a} (batch no. DAH-XXVIII-50, purity 86.9%), 8a -oxo MAB_{1a} (batch no. DAH-XXVIII-2, purity 84.8%), 8a -oxo MAB_{1a} (batch no. DAH-XXVIII-2, purity 84.2%), MSB_{1a} (batch no. JAK-XV-71, purity 86.0%), AB_{1a} (batch no. TEW-V-25, purity 88.4%), AB_{1a} (batch no. TEW-V-25, purity 88.6%), MFB_{1a} (batch no. WFH-IX-89-2, purity 83.8%), aglycone (batch no. DAH-XXVI-26-2, purity 95.2%), aglycone (batch no. DAH-XXVI-26-2, purity 85.1%), FAB_{1a} (batch no. WFH-IX-88-1, purity 77.9%), FAB_{1a} (batch no. WFH-IX-88-2, purity 75.2%) were only used for qualitative purposes.

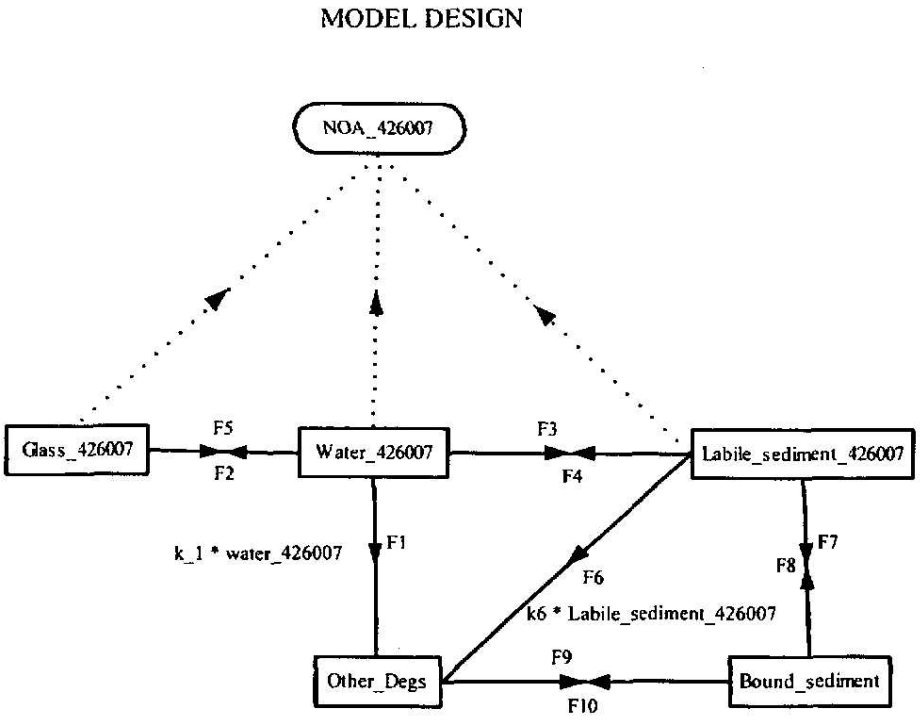
LOD ranged from 0.00008 µg to 0.0004 µg for all assay types.

Calculations. Half-life calculations were performed using Excel 97® and Origin v6.0 and confirmed by Modelmaker v4.0. For half-life determination in experiment 1, the percent of dose for parent was combined with the percent total dose from extracts 1-2 and with the radiocarbon removed from the bottles rinses and stir bar rinses. After day 7, adhesion was no longer a problem due to increased binding to sediment.

Degradation of emamectin benzoate B_{1a}, simultaneous formation and decline of metabolites and formation of CO_2 and bound residues was modelled in ModelMaker 3.0. Half-lives were determined by biphasic exponential calculation in order to more accurately account for the binding of the test substance in the total system after the initial rapid dissipation from the water phase.

Two predominant metabolites zones (10.1 and 9.0%) were summed with the other metabolites in Modelmaker in order to minimise the degrees of freedom in the model, while still accounting for parent binding to sediment and/or glass. Proposed ModelMaker scheme is shown in Figure B.8.4.4.-1.

Figure B.8.4.4-1. Modelmaker analysis of parent in experiment 1.



K_1 = 0.17 Degradation rate constant of NOA-426007 in water fraction
K6 = 0.009 Degradation rate constant of NOA-426007 in sediment fraction that is in equilibrium with
the aqueous phase.

Marquardt Optimization: Ordinary Least Squares
Degrees of freedom for the model: 9
R^2 0.85

For experiment 2, bottle rinses, stir bar rinses and aqueous radioassays were summed to obtain the percent of dose parent.

Results

Experiment 1 (sediment/river water set): Redox potential 165 - 240 mV (water, measured immediately above the sediment layer), DO 24.7 - 126% O_2.

Total accountable radiocarbon ranged from 91.71 to 98.78%.

Experiment 2 (control water set):

Redox potential 147.16 - 317.90 mV (water), DO 11.0 – 129.0% O_2.

Total accountable radiocarbon ranged from 87.77 to 109.63%.

Distribution of radioactivity and parent compound for the aerobic water/sediment and water only incubation is given in table 4.1.4.3-1 and 4.1.4.3-2, respectively. Organic volatiles were < LOD.

Table 4.1.4.3-1. Distribution of radioactivity and parent compound in sandy (River) after **aerobic** incubation (experiment 1). All values represent % of AR.

Compartment/fraction	incubation period (d)									
	0	3	7	14	21	30	45	58	80	105
Water										
total radioactivity	**83.25**	16.04	19.43	15.21	19.52	21.61	16.34	16.00	14.55	18.77
Sediment										
Extractable	6.57	43.66	52.11	71.00	56.51	57.84	**58.51**	52.54	58.11	47.16
Non-extractable	0.30	0.64	0.69	2.35	**6.79**	3.93	3.65	5.04	5.75	6.03
CO_2	n.a.	0.03	0.15	1.67	2.42	4.74	5.39	7.35	9.74	**15.95**
Adhered to bottle and stir bar										
total	7.32	**34.05**	18.49	7.91	13.19	3.56	3.58	3.18	2.07	2.32
Total	97.43	94.41	92.16	98.12	98.42	97.50	93.05	91.71	98.18	98.78
emamectin B_{1a}										
water	76.70	6.90	3.40	1.50	2.40	0.90	1.20	2.10	2.00	0.50
sediment (extractable)	5.70	37.00	37.20	44.50	25.90	27.00	**29.20**	17.30	22.00	13.50
adhered to bottle and stir bar	6.70	25.60	10.20	2.30	6.90	0.70	0.50	0.50	0.20	0.60
total system	89.10	69.50	50.80	48.30	35.20	28.60	30.90	19.90	24.20	14.60

n.a.: not analysed

Table 4.1.4.3-2. Distribution of radioactivity and parent compound in water after **aerobic** incubation (experiment 2). All values represent % of AR.

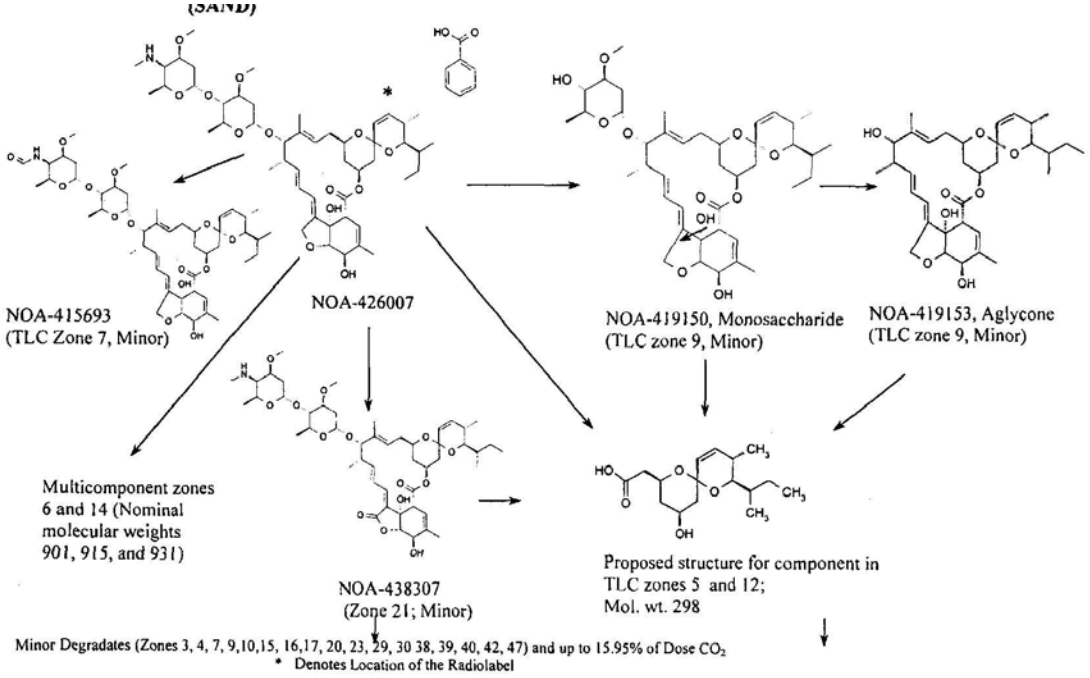
Compartment/fraction	incubation period (d)									
	0	3	7	14	21	30	45	58	80	105
Water										
total radioactivity	94.09	52.28	79.83	73.57	73.29	83.28	73.70	65.14	74.42	**98.91**
CO_2	n.a.	0.05	0.7	0.73	0.34	1.31	1.60	1.03	**2.90**	2.89
Adhered to bottle and stir bar										
total	5.62	**41.77**	25.05	21.15	18.55	15.63	18.40	22.69	10.46	7.84
Total	99.71	94.09	105.57	95.44	92.17	100.21	93.69	88.85	87.77	109.63
emamectin B_{1a}										
water	90.10	41.50	29.00	20.20	16.80	26.10	24.00	24.30	16.50	31.30
adhered to bottle and stir bar	4.90	27.80	12.80	11.10	7.30	0.30	7.80	8.30	3.90	2.60
total system	95.00	69.30	41.80	31.30	24.10	26.40	31.80	32.60	20.40	33.90

n.a.: not analysed

Metabolites. 2D-TLC showed numerous (minor) degradates in both experiments. Relevant metabolites from HPLC analysis are summarised in the remarks.

A suggested pathway is shown in Figure B.8.4.4-2.

Figure 4.1.4.3-1. Proposed degradation pathway in aquatic sediment (sand).



DT_{50}-values as estimated by the author are presented in Table 4.1.4.3-3 (DT_{90} values were not given).

Table 4.1.4.3-3. DT_{50}-values for emamectin benzoate B_{1a} and metabolites.

System	Conditions	Compound	Compartment	DT_{50} [d]	r^2
sand (River)	aerobic	emamectin B_{1a}	water	0.5	0.995
			sediment	77	0.983
			total system	8	0.983
Control (water)	aerobic	emamectin B_{1a}	water	4	0.95
			adhered to glass	577	0.95

Remarks

Redox potential was well below 200 (not aerobic) on one occasion (day 0 replicate B). This will not have affected outcome of the experiment.

For several reference compounds different purities were given for the same batch number. This should be explained by the applicant.

LOD is given in µg.

The low sediment content in the test vessels might be an explanation for the high adherence of the test substance to the walls of the test vessels or to the stir bean(up to 34% in the water/sediment system) when compared to study 1 (max. 0.3%, see above).

The authors suggest using a bi-phasic model. However, this is not acceptable. Since a significant amount of the test substance adhered to the glass wall of the test vessels or to the stir bar (2.07-34.05% and 5.62-

41.77% in the water/sediment and water-only system, respectively), a reliable DT_{50} (degradation) cannot be calculated.

As far as the metabolites are concerned:

Experiment 1: Based on HPLC quantitation, several peaks (degradates) were observed. **Average** peaks that were >5% at two subsequent time steps or >10% at one time step in either water phase or sediment:

Water phase:

- Peak 6: 8.81, 14.57, 12.59, 10.68 and 9.88% on day 21, 30, 45, 58 and 80, respectively.
- *Sediment:*
- Peak 2: 9.57%, 5.58 and 6.73% on day 58, 80 and 105
- Peak 6: 5.96, 7.43, 10.22, 11.57, 13.38 and 9.51% on day 14, 21, 30, 45, 58 and 80, respectively.

It has to be noted that several individual measurements were >5%, but the average of the duplicate measurements was not. Peak 2 was concluded to be 8a -oxo MAB_{1a}. According to the authors, peak 6 was shown to be multicomponent by 2D-TLC and mass spectrometry. This is considered acceptable.

Experiment 2: Based on HPLC quantitation, several peaks (degradates) were observed. **Average** peaks that were >5% at two subsequent time steps or >10% at one time step in the water phase:

- Peak 2: 9.87, 10.6, 9.37, 11.83 (single measurement) and 6.83% on day 7, 14, 21, 30 and 45, respectively. Water phase was not analysed on day 58 and 80. On day 105 the amount of peak 2 was 24.28% (single measurement).
- Peak 6: 10.46, 11.73, 10.88, 5.68 (single measurement) and 14.02% on day 7, 14, 21, 30 and 45, respectively. Water phase was not analysed on day 58 and 80. On day 105 the amount of peak 6 was < minimum quantifiable amount (single measurement).
- Peak 7: 8.43, 7.42 on day 7 and 14.

On day 105 peaks 3, 7 and 8 were also >5% (7.03, 8.33, 5.48 and 5.25%, respectively, all single measurements).

Peak 2 was concluded to be 8a -oxo MAB_{1a}. According to the authors, peak 6 was shown to be multicomponent by 2D-TLC and mass spectrometry. This is considered acceptable. The other peaks were not identified. These did not occur in the water sediment test but only in the water only test.

4.1.4.4 Study 4 - Route and rate of degradation in soil - Aerobic degradation

Study reference: Hand, L.H. and Fleming, E.A. (2006) STUDY IIA, 7.1.1/001

Detailed study summary and results

Reference/notifier	:	Hand, L.H. and Fleming, E.A. (2006)	GLP statement	:	yes
Type of study	:	degradation in soil	Guideline	:	95/36/EC amending Council Directive 91/414/EEC, SETAC-EUROPE Procedures Section 1.1, OECD 307
Year of execution	:	2004	Acceptability	:	acceptable

Test substance	:	[^{14}C]-NOA426007 (emamectin benzoate B_{1a}), radiochemical purity > 98%

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH	pF	Duration [d]	DT_{50} [d]
[^{14}C]-emamectin benzoate B_{1a}	sandy clay loam	aerobic	0.13	20	4.8	5.8	2.5	120	45.9
[^{14}C]-emamectin benzoate B_{1a}	loam	aerobic	0.13	20	5.4	7.4	2.5	120	25.2
[^{14}C]-emamectin benzoate B_{1a}	silty clay loam	aerobic	0.13	20	1.8	8.1	2.5	120	414

Description

Soil.

Soil from the locations 18 Acres (Berkshire, UK), Gartenacker (Les Barges/VS, Switzerland), and Marsillargues (Marsillagues, France was air dried, 2 mm sieved.Soil characterisation is reported

Reported soil classifications are according to USDA soil Triangle. Soil characteristics are reported in table 4.1.4.5-1. Biomass determined according to Anderson and Domsch (1978) at start and end of the study. Biomass contents where as reported in table 4.1.4.5-2.

Table 4.1.4.5: Soil characteristics

Property	Reported Value (Reference 6)		
	18 Acres	Gartenacker	Marsillargues
pH in water [(a)]	5.8	7.4	8.1
pH in 0.01M $CaCl_2$ [(b)]	5.3	6.7	7.4
% Sand (0.05-2.0 mm)	56	47	9
% Silt (0.002-0.05 mm)	20	42	58
% Clay (<0.002 mm)	24	11	33
% Organic Matter [(c)]	4.8	5.4	1.8
% Organic Carbon [(c)]	2.8	3.1	1.0
Cation Exchange Capacity (meq/ 100 g) [(d)]	22.5	12.0	12.3
Moisture Holding Capacity 0.1 Bar (% Moisture Content) [(e)]	31.6	43.3	27.5 [(k)]
Moisture Holding Capacity 0.33 Bar (% Moisture Content) [(f)]	23.4	31.1	29.4 [(k)]
Moisture Holding Capacity 15 Bar (% Moisture Content) [(f)]	10.7	10.4	16.1
Available Phosphorus (mg/L) [(g)]	10.2	52.4	24.8
Available Potassium (mg/L) [(h)]	126	96	305
Available Magnesium (mg/L) [(i)]	122	67	275
Total Nitrogen (% w/w) [(j)]	0.238	0.305	0.135
Textural Classification (USDA Soil Triangle)	Sandy Clay Loam	Loam	Silty Clay Loam

(a) pH by glass junction electrode in a 1:2 soil : water slurry.
(b) pH by glass junction electrode electrode in a 1:2 soil : 0.01M $CaCl_2$ slurry
(c) Organic matter using potassium dichromate to oxidise the organic matter, followed by titration of excess dichromate with ferrous sulphate (Walkley-Black method). A factor of 1.724 was used to convert organic matter to organic carbon.
(d) Cation exchange capacity by sodium saturation at pH 7 and flame photometry.
(e) Moisture holding capacity using a porous plate (Haines) apparatus (expressed as % w/w air-dried soil).
(f) Moisture holding capacity using a ceramic pressure plate technique (expressed as % w/w air-dried soil).
(g) Using "Olsen's Extraction", i.e. extraction with sodium bicarbonate, reaction with acid ammonium molybdate. This forms a blue complex, the colour of which is measured spectrophotometrically at 880 nm.
(h) By extraction with ammonium nitrate and analysis by flame photometry
(i) By extraction with ammonium nitrate and analysis by atomic absorption spectrophotometry
(j) Using the "Dumas Technique", i.e. combustion using a Total Combustion Analyser.
(k) Due to the high clay content of this soil, increase of the pressure from 0.1 Bar to 0.33 Bar is likely to have caused some blockage in the apparatus with fine particles. Consequently, there is no significant difference between the measured moisture holding capacities at 0.1 and 0.33 Bar.

Table 4.1.4.5-2: microbial biomass

Soil Name	Initial Microbial Biomass Carbon (mg/100g Soil)	Initial % Microbial Biomass Carbon [(a)]	Final Microbial Biomass Carbon (mg/100g Soil)	Final % Microbial Biomass Carbon [(a)]
18 Acres	30.32	1.09	39.94	1.50
Gartenacker	47.46	1.41	62.39	1.96
Marsillargues	37.68	3.61	26.28	2.52

(a) Expressed as % of the total organic carbon content of the soil

.

No pesticide or fertiliser treatments took place on all plots for at least 12 months prior to the study.

Method. (23-^{14}C)-emamectin benzoate B_{1a} (purity 98.4%) was applied to the soil, application rate 0.13 mg/kg. Aerobic incubation, final moisture content approaching pF 2: 28.5, 34.9 and 20.7% for sandy clay loam, loam and silty clay loam soil, respectively. Incubation at 20 ± 2 °C in the dark, ventilation with moistened air. Effluent air passed through volatile traps (2 M NaOH). Duplicate soil samples taken at 0, 7, 14, 21, 28, 60, 90 and 120 days.

Analysis. Extraction with acetonitrile/0.1 M ammonium acetate (8:2 v/v) repeated two to four times, extracts were combined (extract 1). The entire extraction procedure was repeated exactly (extract 2) only for the day 0 and day 7 samples. Analysis of extracts by LSC and HPLC-UV (245 nm). Selected extracts were also analysed by 2D-TLC. Bound residues determined by LSC after combustion. Volatiles analysed by LSC, CO_2 confirmed by $BaCO_3$-precipitation.

Reference compounds for HPLC and TLC: emamectin benzoate B_{1a}, FAB_{1a}, MSB_{1a}, aglycone, 8a-oxo MAB_{1a}, AB_{1a}, 8,9-Z-MAB_{1a}, MFB_{1a} and 8-OH MAB_{1a}. Procedural recoveries HPLC 90-110%. Identification of metabolites by LC-MS/MS and LC-NMR.

Calculations. Rate constants for degradation of emamectin benzoate B_{1b} and concurrent formation and decline of metabolites, CO_2 and bound residues were calculated using both a simple first order (SFO) and first order multi-compartment kinetics (FOMC) to the parent data.

Results

Microbial biomass at end was 399.4, 623.9 and 262.8 mg C/kg for the sandy clay loam, loam and silty clay loam soils, respectively.

The mean mass balance was 94.2, 94.9 and 98.3% of the applied radioactivity (AR) for sandy clay loam, loam and silty clay loam, respectively. Mineralisation of emamectin benzoate B_{1b} occurred in all soils, and levels of radiolabelled carbon dioxide accumulated to 7.8%, 8.8% and 1.3% AR in the respective soils.

Aerobic incubation: Distribution of radioactivity for aerobic incubation is given in Table 4.1.4.5-3. Maximum levels are indicated in bold. Distribution of radioactivity as emamectin benzoate B_{1a} is given in Table 4.1.4.5-4. Distribution of applied radioactivity of metabolites of emamectin benzoate B_{1a} is given in Table 4.1.4.5-5.

Table 4.1.4.5-3. Distribution of radioactivity after aerobic incubation of emamectin benzoate B_{1a}. All values in % of AR.

Time [d]	sandy clay loam				loam				silty clay loam			
	Total extracted	Bound residues	CO_2	Recovery	Total extracted	Bound residues	CO_2	Recovery	Total extracted	Bound residues	CO_2	Recovery
0	97.3	2.8	0.0	100.1	95.3	4.1	0.0	99.3	97.2	1.5	0.0	98.6
7	89.0	9.0	0.2	98.2	93.0	8.1	0.2	101.3	88.8	2.6	0.0	91.4
14	83.0	10.8	0.7	94.5	86.6	10.8	0.7	98.1	95.0	1.9	0.0	97.0
21	77.3	21.1	1.1	99.4	79.8	12.3	1.4	93.4	98.6	1.6	0.1	100.2
28	74.8	15.0	1.5	91.2	79.8	15.3	2.1	97.2	102.1	1.5	0.2	103.7
60	66.9	19.5	3.5	89.8	66.7	22.0	4.6	93.2	94.5	2.6	0.4	97.4
90	57.7	**33.6**	5.8	97.0	56.9	27.1	6.9	90.9	95.5	4.2	0.8	100.4
120	56.2	19.6	7.8	83.5	47.3	**29.6**	8.8	85.7	91.2	**5.5**	1.3	97.9

Distribution of applied radioactivity as emamectin benzoate B_{1b} is given in Table 8.1.1.1-4.

Table 4.1.4.5-4. Distribution of applied radioactivity as emamectin benzoate B_{1a}. All values in % of AR.

Time [d]	sandy clay loam	loam	silty clay loam
0	95.7	93.5	96.2
7	78.4	79.9	84.8
14	63.9	56.6	91.7
21	53.4	45.4	93.9
28	48.8	40.4	92.0
60	37.9	19.7	86.7
90	22.6	16.2	78.3
120	24.9	8.4	76.7

Nine degradates were observed in the study. Metabolite 1 was identified as 8a-OH MAB_{1a} (after analyses by LC-MS/MS), metabolite 2 was identified as AB_{1a}, metabolite 3 was identified as 8a -oxo MAB_{1a} and metabolite 8 was identified as aglycone. The remaining metabolites did not co-chromatograph with any of the reference standards available.

Table 4.1.4.5-5. Distribution of applied radioactivity of metabolites of emamectin benzoate B_{1a}. All values in % of AR of emamectin benzoate B_{1a}

soil	Time	Metabolite									
	[d]	1*	2	3	4	5	6	7	8	9	unretained
18 Acres, sandy clay loam	0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
	7	1.9	0.4	**1.5**	1.7	0.3	1.0	0.1	0.4	0.0	0.9
	14	2.5	0.9	1.3	2.2	0.9	0.9	0.9	1.0	0.6	1.1
	21	2.2	**1.2**	1.4	**3.8**	1.8	2.2	0.1	1.3	1.2	0.4
	28	**4.7**	0.5	1.4	1.0	2.3	2.0	1.4	0.7	1.1	1.4
	60	2.0	0.2	0.4	0.0	1.8	2.1	2.1	1.0	**2.3**	5.3
	90	2.0	0.0	0.7	0.1	**2.8**	**3.5**	**3.5**	1.1	2.0	3.6
	120	1.6	0.3	0.8	0.1	2.0	1.5	1.4	**2.0**	1.2	**9.1**
Gartenacker, loam	0	0.6	0.0	0.0	ND	0.0	0.0	0.0	0.0	0.0	0.0
	7	6.6	0.5	1.5	ND	0.2	0.0	0.3	0.1	0.0	0.6
	14	12.9	1.5	4.0	ND	1.1	0.1	0.8	0.7	0.6	2.3
	21	**13.8**	**1.8**	3.7	ND	2.2	0.0	1.4	0.0	0.8	1.7
	28	12.5	1.3	**4.5**	ND	2.5	1.3	1.5	0.8	1.3	4.8
	60	9.7	0.5	2.5	ND	3.6	1.0	**3.7**	**1.7**	**2.8**	5.9
	90	6.8	0.0	1.5	ND	3.3	1.2	1.8	0.9	2.0	**6.2**
	120	4.8	0.0	1.0	ND	**3.8**	**2.4**	2.0	1.3	2.0	3.3
Marsillargues, silty clay loam	0	0.0	0.0	0.0	ND	ND	ND	ND	ND	ND	0.0
	7	2.0	0.0	0.3	ND	ND	ND	ND	ND	ND	0.1
	14	1.1	0.0	**0.6**	ND	ND	ND	ND	ND	ND	0.0
	21	2.1	0.5	0.2	ND	ND	ND	ND	ND	ND	0.0
	28	2.6	0.3	**0.6**	ND	ND	ND	ND	ND	ND	0.0
	60	1.9	0.4	0.2	ND	ND	ND	ND	ND	ND	0.0
	90	**4.9**	0.0	0.0	ND	ND	ND	ND	ND	ND	0.0
	120	4.3	**0.7**	0.5	ND	ND	ND	ND	ND	ND	**2.5**

* metabolite 1 consisted of 8a-OH MAB_{1a} and a number of other minor metabolites

After further analysing the major peak of metabolite 1 from Gartenacker soil, by analysing a soil extract from an exaggerated rate treatment, it turned out that three components were present in the respective fraction. Nuclear magnetic resonance (NMR) analysis of selected extracts indicated that the major portion (approximately 75%) of the metabolite 1 fraction represented 8a-OH MAB_{1a}, the remaining two minor components could not be identified. Additional analysis of day 14, day 21 and day 28 samples with an optimised HPLC system resulted in a slight change of radioactivity levels of metabolite 1 compared to the results of Table 4.1.4.5-5. The additional analysis showed the peak of metabolite 1 on day 14 with 11.7 % AR, the majority of this peak was 8a-OH MAB_{1a} with 9.3 % of AR. Therefore the results for metabolite 1 as

reported in Table 4.1.4.5-5 are corrected with a factor of 0.79 resulting in a maximum formation percentage of metabolite 1 of 10.9% (day 21, Gartenacker soil).

The 90 DAT samples of 18 Acres and Gartenacker soil still contained significant levels of radioactivity (25 to 35% AR) after the extraction with organic solvents. Additional extractions were performed to investigate the nature of the unextracted residues. Unextracted residues were demonstrated to be associated with surface-sorbed metal ions in addition to partitioning into organic matter. In 18 Acres soil, no significant levels of radioactivity were associated with the humin fraction. Between 2.1 and 3.5% of AR was associated with the fulvic fraction, 6.8 to 7.5% was asssociated with the humic fraction.

In Gartenacker, 2.4-5.6% of AR was associated with the fulvic fraction, and 3.5-6.7% was associated with the humic acid fraction.

DT_{50}-values as reported by the author are shown in Table 4.1.4.5-6.

Table 4.1.4.5-6. DT_{50}-values for emamectin benzoate B_{1a} reported by author

Compound	Model used	Soil type	DT_{50} [d]	(r^2)
emamectin benzoate B_{1a}	SFO	sandy clay loam	46	0.91
		loam	25	0.97
		silty clay loam	348	0.96
emamectin benzoate B_{1a}	FOMC	sandy clay loam	30	0.99
emamectin benzoate B_{1a}		loam	21	0.99
emamectin benzoate B_{1a}		silty clay loam	348	0.96

Remarks

N-nitroso MAB_{1a} was not used as reference substance. Gartenacker soil not sieved. Soil history only reported for the last year.

DT_{50}-values for emamectin benzoate B_{1a} are recalculated by RMS regarding the principles of FOCUS Kinetics. The results of the recalculated values are given in Table 4.1.4.5-7:

Table 4.1.4.5-7. Recalculated DT_{50}-values for emamectin benzoate B_{1a}.

Compound	Soil type	DT_{50} [d] SFO	R^2	χ^2	DT_{50} [d] FOMC model	R^2	χ^2
emamectin benzoate B_{1a}	sandy clay loam	45.9	0.9129	10.5	30	0.989	4.0
	loam	25.2	0.9698	8.8	21.3	0.99	5.4
	silty clay loam	414	0.7393	3.1	No adequate fit		

The DT_{50} value for silty clay loam is an extrapolated value; at the end of the test 76.7% of AR was still present as emamectin benzoate B_{1a}. Visual inspection of the SFO fit and distribution of residuals showed adequate fitting results.

4.1.4.5 Study 5 - Route and rate of degradation in soil - Aerobic degradation

Study reference: Jungmann V., Nicollier, G. (2006), STUDY IIA, 7.1.1/002

Detailed study summary and results

Reference/notifier	:	Jungmann V., Nicollier, G. (2006)	GLP statement	:	yes
Type of study	:	degradation in soil	Guideline	:	95/36/EC amending Council Directive 91/414/EEC, SETAC-EUROPE Procedures Section 1.1, OECD 307
Year of execution	:	2006	Acceptability	:	acceptable
Test substance	:	[^{14}C]-NOA426007 (emamectin benzoate B_{1a}), radiochemical purity > 99.9%			

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH [KCl]	pF	Duration [d]	DT50 [d]
[^{14}C]- emamectin benzoate B_{1a}	silt loam	aerobic[1]	0.031	19.6 ± 0.5	4.6	7.08	2.2	119	39.3
[^{14}C]- emamectin benzoate B_{1a}	silt loam	aerobic[2]	0.031	19.6 ± 0.5	4.6	7.08	3.5*	119	98.1[2] 53.7[1]
[^{14}C]- emamectin benzoate B_{1a}	silt loam	aerobic[1]	0.31	19.6 ± 0.5	4.6	7.08	2.2	119	32.4

[1]at a soil moisture of 40% MWC
[2]at a soil moisture of 20% MWC
*estimated value

Methods

Test type: degradation in soil. The study is performed according to 95/36/EC amending Council Directive 91/414/EEC, SETAC-EUROPE Procedures Section 1.1, OECD 307 and is GLP compliant. The study is considered acceptable.

Soil. Silt loam (Gartenacker, CH) was air dried and 2 mm sieved. Collection of the soil was performed according to ISO guideline 10381-6. The soil has not been treated during the previous year in any way that could severely affect the microbial populations.

Table 4.1.4.6-1: soil properties.

Origin	Gartenacker
Batch No.	10.06.05
Classification (USDA)	Silt loam
pH (KCl)	7.08
$CaCO_3$ (%)	8.0
C_{org}(%)	2.7
N_{tot} (%)	0.25
CEC (mmole/Z/100 g soil)	15.5
Bulk density (g/ml)	1.20
Composition:	
Clay (%)	9.5
Silt (%)	50.6
Sand (%)	39.9
pF 2 (g H_2O / 100 g dry soil)	32.2
MWC (100%) (g H_2O / 100 g dry soil) g	68.0
MWC (40%) (g H_2O / 100 g dry soil)	27.2
Microbial biomass (mg C/100 g soil) day 0 day 119 (Test 1+3) day 119 (Test 2)	80.8 50.1 51.5

Method. (23-^{14}C)- emamectin benzoate B_{1a} was dispersed in acetonitrile and applied to the soil surface, application rates 0.031 (test 1 and 2) and 0.31 (test 3) mg as/kg. The soil was adjusted to the appropriate

moisture content (40% MWC test 1 and 3, 20% test 2). Incubation at 19.6 ± 0.5 °C in the dark under continuous ventilation with moistened air. The effluent gas was passed through a trapping system. In addition, for identification purposes of unknown metabolites, four replicates were treated with 14.5 mg as/kg soil. Duplicate soil samples were taken at 0, 3, 7, 14, 28, 56, 91 and 119 days after treatment. Microbial biomass was determined at start and end according to Anderson and Domsch (1978).

Analysis. Extraction at room temperature with acetonitrile/water 4:1 (v/v) repeated up to four times and the supernatants of all extraction steps were combined (= Extract 1). The soil was further extracted with acetonitrile under reflux. Since the radioactivity in the extract was generally not greater than 3.1% of the applied radioactivity (AR) with the exception of replicate A at 56 DAT in Test 1 (5.1% AR), the extract was combined with Extract 1.

Additional harsh extraction of day-119 samples by reflux with acetonitrile/0.1 N HCl (4:1). After centrifugation, the supernatant was decanted and the soil was subjected to organic matter fractionation. An aliquot of 10 g soil was extracted using 0.5N $NaOH_{(aq)}$. After centrifugation, the supernatant was decanted and acidified to pH 1 with HCl. The supernatant containing the fulvic acid fraction was decanted and the remaining solid (humic acid fraction) was dissolved in 0.5N NaOH. The radioactivity associated with the humin fraction in the soil was calculated by subtraction of the fulvic and humic acid fractions from the non-extractable residues in soil after the acidic harsh extraction.

Combined extracts were analysed by LSC. Isolation and purification of fractions containing potential metabolites were done by HPLC or by LC. Corresponding fractions were combined, concentrated and dissolved in acetonitrile. If necessary, further clean-up was done by means of HPLC prior to HPLC-NMR and MS analysis.

Calculations. Rate constants for degradation of [^{14}C] -emamectin benzoate B_{1a} were calculated assuming simple first order kinetics (SFO) or first order two compartment kinetics (FOTC). DT_{50} values of metabolites were not reported.

Results

The actual application rate was 0.031 and 0.307 mg as/kg dry soil in test 1/2 and test 3, respectively.

Microbial biomass at end 50.1 (test 1 and 3) and 51.5 (test 2) mg C/100 g. Total mass balance for replicate samples during the test period ranged from 97.9 to 109.3% AR for test 1, from 104.1 to 108.4% AR for test 2, and from 99.5 to 106.9% AR for test 3. Carbon dioxide accounted for 1.7% AR (test 2) to 5.2% AR (test 3) at 119 DAT. Organic matter fractionation showed most of the remaining radioactivity associated with the humic acid fraction (8 to 13% AR) and approximately 5 to 7% associated each with the fulvic acid and the humin fraction.

Table 4.1.4.6-2. Distribution of radioactivity after aerobic incubation of (23-^{14}C)-emamectin benzoate B_{1a} to Gartenacker soil. All values in % of AR.

DAT	Repl.	CO_2	Extract 1	Reflux	Total extract	emamectin benzoate B_{1a}	8a-OH MAB_{1a}	8a - oxo MAB_{1a}	MFB_{1a}	N-nitroso MAB_{1a}	MSB_{1a}	NIR	NER	Total
Test 1: 40% MWC, 0.031 mg ai/kg dry soil														
0	A	n.p.	98.7	1.8	100.5	100.5	0.0	0.0	0.0	0.0	0.0	0.0	1.9	102.4
	B	n.p.	101.5	1.8	103.3	103.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	104.5
3	A	0.2	96.8	1.7	98.6	90.0	4.3	1.6	0.0	0.4	0.0	2.3	4.5	103.3
	B	0.2	98.7	1.7	100.4	90.5	4.7	1.4	0.0	0.4	0.0	3.4	4.0	104.6
7	A	0.3	98.0	2.4	100.4	79.9	7.9	2.1	0.4	0.9	0.0	9.2	5.6	106.4
	B	0.3	97.2	2.6	99.8	82.3	7.0	2.0	0.3	1.3	0.0	6.9	5.3	105.5
14	A	0.7	94.3	2.5	96.8	62.2	7.3	2.0	2.2	11.3	0.0	11.8	8.2	105.7
	B	0.7	92.2	2.3	94.5	58.2	8.0	2.0	2.8	11.3	0.0	12.2	9.8	105.1
28	A	1.2	93.1	2.2	95.3	49.3	7.0	2.3	5.9	14.9	0.0	5.9	8.2	104.8
	B	1.2	91.9	2.2	94.0	45.7	7.8	2.3	**6.2**	**15.7**	0.0	16.3	12.8	108.0
56	A	0.9	87.7	5.1	92.8	38.4	**8.1**	**3.2**	4.7	8.5	4.4	25.5	20.0	113.7
	B	0.9	89.5	2.3	91.8	35.9	8.0	2.7	5.1	9.3	**4.5**	26.3	12.2	104.9
91	A	2.3	76.1	1.3	77.4	23.0	7.0	2.3	5.6	6.3	2.4	30.9	17.6	97.3
	B	2.3	73.9	3.0	76.9	23.9	5.6	2.2	5.3	6.5	2.1	31.3	25.6	104.8
119	A	2.3	71.8	2.7	74.5	21.5	5.3	2.7	3.9	2.7	2.8	35.6	16.9	93.7

DAT	Repl.	CO_2	Extract 1	Reflux	Total extract	emamectin benzoate B_{1a}	8a-OH MAB_{1a}	8a - oxo MAB_{1a}	MFB_{1a}	N-nitroso MAB_{1a}	MSB_{1a}	NIR	NER	Total
	B	2.2	69.1	2.9	72.0	19.4	5.3	2.2	3.2	2.5	2.6	36.8	27.8	102.1
Test 2: 20% MWC, 0.031 mg ai/kg dry soil														
0	A	n.p.	100.2	1.3	101.5	101.5	0.0	0.0	0.0	0.0	0.0	0.0	1.2	102.7
	B	n.p.	102.7	1.4	104.1	104.1	0.0	0.0	0.0	0.0	0.0	0.0	1.3	105.4
3	A	0.1	102.3	2.0	104.2	101.3	1.5	0.6	0.0	0.0	0.0	0.8	1.6	105.8
	B	0.1	100.2	2.1	102.4	99.1	1.3	0.8	0.0	0.0	0.0	1.2	2.2	104.6
7	A	0.6	101.3	2.1	103.4	98.4	2.3	0.9	0.0	0.8	0.0	2.0	3.2	107.3
	B	0.6	100.4	2.0	102.3	93.2	2.1	0.9	0.0	2.4	0.0	3.7	3.2	106.2
14	A	0.9	100.4	1.8	102.2	87.2	4.3	1.3	0.5	6.8	0.0	2.0	6.5	109.7
	B	0.9	99.1	1.8	100.9	87.0	3.7	1.1	1.0	5.5	0.0	2.3	4.4	106.2
28	A	0.4	96.5	2.0	98.5	74.4	4.9	1.4	**3.1**	**11.5**	0.0	3.1	5.6	104.5
	B	0.4	98.1	1.9	100.0	81.2	5.4	2.0	1.9	5.9	0.0	3.6	6.0	106.4
56	A	0.7	90.6	2.3	92.9	62.8	6.6	2.2	1.8	3.9	0.5	15.1	11.2	104.9
	B	0.7	92.2	2.1	94.3	62.1	6.0	2.0	2.9	4.7	0.7	15.9	10.5	105.5
91	A	1.5	89.3	2.5	91.8	54.0	**10.0**	3.0	2.6	3.1	0.0	19.1	15.4	108.7
	B	1.5	90.0	2.4	92.4	59.4	6.7	2.1	3.1	3.8	0.0	17.3	14.1	108.1
119	A	1.7	85.1	2.5	87.6	48.6	7.4	2.2	2.3	3.3	1.2	22.6	18.1	107.5
	B	1.7	80.3	2.6	83.0	41.0	7.7	**3.1**	3.1	3.2	**2.1**	22.8	21.2	105.9
Test 3: 40% MWC, 0.307 mg ai/kg dry soil														
0	A	n.p.	102.4	1.2	103.6	103.6	0.0	0.0	0.0	0.0	0.0	0.0	1.6	105.2
	B	n.p.	103.2	1.4	104.6	104.6	0.0	0.0	0.0	0.0	0.0	0.0	1.6	106.2
3	A	0.1	101.6	1.7	103.3	91.9	6.1	2.7	0.0	0.0	0.0	2.6	3.3	106.7
	B	0.1	100.4	1.3	101.7	92.3	4.8	2.2	0.0	0.0	0.0	2.4	3.4	105.2
7	A	0.2	98.8	2.0	100.8	81.2	8.6	2.5	0.0	0.8	0.0	7.7	5.0	106.0
	B	0.2	98.2	2.1	100.3	80.1	9.1	2.5	0.0	1.3	0.0	7.3	5.8	106.3
14	A	0.4	95.6	1.9	97.5	62.8	9.0	2.8	2.5	**10.1**	0.0	10.3	8.7	106.6
	B	0.4	94.2	1.7	95.9	58.3	8.6	2.7	4.2	**10.1**	0.0	22.0	8.6	104.9
28	A	0.6	82.7	2.7	85.5	46.8	8.3	2.9	3.7	**10.1**	0.0	13.7	10.8	96.9
	B	0.6	83.4	3.1	86.5	43.6	**11.1**	3.0	4.3	8.2	0.0	16.3	14.9	102.1
56	A	1.9	82.8	2.4	85.2	33.1	7.9	2.6	**4.7**	8.0	0.9	30.0	19.6	106.7
	B	1.9	84.0	2.5	86.5	26.8	8.6	**3.5**	4.0	5.6	**2.8**	35.2	18.7	107.1
91	A	3.2	78.2	2.7	80.9	22.9	7.1	3.2	4.4	6.1	2.4	34.8	22.4	106.6
	B	3.3	75.6	2.7	78.3	21.4	7.0	3.2	4.4	4.8	2.3	35.2	23.1	104.7
119	A	5.1	70.8	2.8	73.6	20.2	6.3	2.5	**4.7**	4.9	1.2	33.8	27.1	105.9
	B	5.2	68.5	2.5	71.0	14.5	4.6	1.8	4.2	2.6	2.2	41.1	27.6	103.8

NIR: non identified residu
NER: non extractable residu

Two major metabolites were formed, 8a-OH MAB_{1a} (NOA 438036, max 11.1% in one replicate) and N-nitroso MAB_{1a} (NOA 459720, max. 11.5% in one replicate). MFB_{1a} (NOA 415692) was detected at 2 times >5% in one test.

In the original study the author calculated DT_{50} and DT_{90} values for the active substance only using SFO and FOMC regression. In those calculations FOMC appeared to have better fit to the data. In an addendum to the report DT_{50}- and DT_{90}- values were re-calculated by the notifier following the recommendations of the FOCUS Kinetics guidance document. The software package ModelManager was used with SFO kinetics, fitting parent and one metabolite simultaneously. All fits showed acceptable visual fitting. Resiudal plots were acceptable. $\chi 2$ values are reported in table 4.1.4.6-3.

Table 4.1.4.6-3. DT_{50}-values for emamectin benzoate B_{1a} and metabolites.

Compound	Test	Conditions	Single first order				
			DT_{50} [d]	DT_{90} [d]	χ^2	R^2	ff
emamectin benzoate B_{1b}	1	0.031 mg as/kg, 40% MWC	39.3	130	10.1	0.97	-
emamectin benzoate B_{1b}	2	0.031 mg as/kg, 20% MWC	98.1	326	3.2	0.96	-
emamectin benzoate B_{1b}	3	0.307 mg as/kg, 40% MWC	32.4	107	10.1	0.97	-
NOA 459720	1	0.031 mg as/kg, 40% MWC	14.1	46.8	35.2	0.97	0.65
NOA 459720	2	0.031 mg as/kg, 20% MWC	8.2	27.2	35.8	0.69	0.08

Compound	Test	Conditions	Single first order				
NOA 459720	3	0.307 mg as/kg, 40% MWC	19	63.2	34.5	0.97	0.35
NOA 438306	1	0.031 mg as/kg, 40% MWC	14	46.5	24.6	0.97	0.54
NOA 438306	2	0.031 mg as/kg, 20% MWC	36.6	122	11.6	0.92	0.38
NOA 438306	3	0.307 mg as/kg, 40% MWC	15.3	50.9	24.5	0.973	0.54
NOA 415692	1	0.031 mg as/kg, 40% MWC	39.4	131	24.9	0.976	0.16
NOA 415692	2	0.031 mg as/kg, 20% MWC	nc	nc	nc	nc	nc
NOA 415692	3	0.307 mg as/kg, 40% MWC	74	246	22.3	0.977	0.10

nc: not calculated

From the visual fit of parent curve it appeared that SFO did not fit the data accurately. Simultaneous fitting of parent and 1 metabolite using FOMC for parent and SFO for metabolite fit gave a better visual fit to the data. Residual plots were acceptable for the parent FOMC fit. For metabolites the residuals were not homogeneously distributed in all cases.

Table 4.1.4.6--4. DT_{50}-values for emamectin benzoate B_{1a} and metabolites.

Compound	Test	Conditions	FOMC/SFO fitting				
			DT_{50} [d]	DT_{90} [d]	χ^2	R^2	ff
emamectin benzoate B_{1b}	1	0.031 mg as/kg, 40% MWC	25.1	329	3.4	0.996	-
emamectin benzoate B_{1b}	2	0.031 mg as/kg, 20% MWC	97.1	1712	1.9	0.98	-
emamectin benzoate B_{1b}	3	0.307 mg as/kg, 40% MWC	21.4	224	1.9	0.996	-
NOA 459720	1	0.031 mg as/kg, 40% MWC	25.1	46.8	35.2	0.97	0.65
NOA 459720	2	0.031 mg as/kg, 20% MWC	[1]nc	nc	nc	nc	nc
NOA 459720	3	0.307 mg as/kg, 40% MWC	41.2	137	36.7	0.996	0.19
NOA 438306	1	0.031 mg as/kg, 40% MWC	24.3	356	19.6	0.996	0.23
NOA 438306	2	0.031 mg as/kg, 20% MWC	98.6	328	7.8	0.997	0.23
NOA 438306	3	0.307 mg as/kg, 40% MWC	35.8	119	17	0.997	0.26
NOA 415692	1	0.031 mg as/kg, 40% MWC	[1]nc	nc	nc	nc	nc
NOA 415692	2	0.031 mg as/kg, 20% MWC	nc	nc	nc	nc	nc
NOA 415692	3	0.307 mg as/kg, 40% MWC	356	1182	24.1	0.998	0.06

[1] ModelMaker FOMC run reports error. Model does not fit the data

For parent FOMC fitted better to the data than SFO. The combination on FOMC fitting for the parent and SFO fitting for metabolite gave no better fit for the metabolites.

As the SFO fitting appeared to be good enough based on visual fit and χ^2, SFO will be used for modeling.

Remarks

Soil history not clear, described as "has not been treated during the previous year in any way that could severely affect the microbial populations". No history on pesticide use. From the DT_{50}-values for emamectin benzoate B_{1a} it is concluded that a lower moisture content of the soil resulted in a higher DT_{50} value, there was no qualitative effect of the lower soil moisture content.

4.1.4.6 Study 7 - Route and rate of degradation in soil - Aerobic degradation

Study reference: Clark, A. (2003), STUDY IIA, 7.1.1/003

Detailed study summary and results

Reference/notifier	:	Clark, A. (2003)	GLP statement	:	yes
Type of study	:	degradation in soil	Guideline	:	Subdivision N, Section 162-1, 1982
Year of execution	:	2002	Acceptability	:	acceptable

Test substance : [23-^{14}C]-NOA426007 (emamectin benzoate B_{1a}), radiochemical purity 98.6 %, [23-^{14}C]-NOA422390 (emamectin benzoate B_{1b}), purity 98.7%

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH [KCl]	pF	Duration [d]	DT50 [d]
[^{14}C]- emamectin benzoate B_{1a}	sandy loam	aerobic[1]	0.015	25	0.5	8.3	2.2	100	63.7
[^{14}C]- emamectin benzoate B_{1b}	sandy loam	aerobic[1]	0.015	25	0.5	8.3	2.2	100	71.6

Methods:

Soil. Sandy loam (Pinal County, Arizona): CEC 177 mmol/kg, field moisture capacity at 1/3 bar 16.0%, bulk density 1.46 g/cm^3, microbial biomass 99.9 mg C/kg soil. Microbial biomass determined at end according to Anderson and Domsch (1978). Soil was 2-mm sieved, adjusted to approximately 75% of field moisture capacity and incubated for one week at 25 ±1°C. No pesticides have been used from 1998-2002.

Method. The degradation of both components of emamectin benzoate (test substances: ^{14}C-MAB_{1a} and ^{14}C-MAB_{1b}) under aerobic conditions was investigated in parallel in Arizona sandy loam soil for 100 days. The test substances were evenly applied to the soil as a solution in ethanol at a nominal concentration of 0.15 (experiment 1 and 2) and at an exaggerated concentration of 4.9-5.6 mg/kg dry soil (experiment 3 and 4) for potential identification work. Samples were incubated in aerated systems in the dark at 25°C. Effluent air passed through volatile traps (KOH). Duplicate test flasks from experiments 1 and 2 were sampled at 0, 2, 4, 7, 14, 21, 30, 60 and 100 days and for experiments 3 and 4 on days 0, 30 and 60. Experiments 3 and 4 were conducted for potential identification.

Analysis. Two times extraction with 4:1 acetonitrile/water (100 mM ammonium acetate) extracts combined and analysed by LSC, 2D-TLC and HPLC. From day 4 onwrds soils were further extracted with 3:1 acetonitrile: acidified water (0.5 N HCl, pH 6) twice and the two extracts combined. The combined extract was analysed as described before.

Each sample from days 30 to 100 was further subjected to organic matter fractionation to characterise the humin, fulvic acid, and humic acid binding profile to the ^{14}C components. The soil was extracted with 0.5N NaOH. The remaining radioactivity in the soil is considered to be associated with the humin fraction. Methanol was added to the supernatant thereby precipitating the humic acid fraction, the fulvic acid fraction remained in the supernatant.

At each step, the volumes of the supernatant were measured and the total radioactivity was quantified by LSC. Post-extraction soils were air-dried, homogenised and combusted and the radioactivity was quantified by LSC. Volatile trapping solutions were radioassayed by LSC.

The limit of detection ranged from 0.00008 to 0.0004 µg and from 0.00007 to 0.0004 µg/L for all assay types for samples dosed with ^{14}C-emamectin B_{1a} and ^{14}C-emamectin B_{1b}, respectively.

Reference substances used were: emamectin B_{1a}, emamectin B_{1b}, 8,9-Z-MAB_{1a}, AB_{1a}, MFB_{1a}, FAB_{1a}, MSB_{1a}, 8a -oxo MAB_{1a}.

Calculations: Rate constants for degradation of emamectin B_{1a} and emamectin B_{1b} were calculated using the following functions: C_0 * Exp ($-K_0$*X), $T_{1/2}$= ln2/slope and with ModelMaker 3.03, assuming first-order kinetics.

Results

Average material balance ranged from 91.53% - 106.99% AR for the kinetic samples of emamectin B_{1a}, 90.03% - 104.14% AR for the emamectin B_{1b} kinetic samples and 94.98% to 108.55% AR for the MAB_{1a} identification samples and 100.63% to 104.75% AR for the MAB_{1b} identification samples.

Microbial biomass at end 128.4 mg C/kg soil. Distribution of radioactivity for respective substances is given in Table 4.1.4.7-1. Maximum levels are indicated in bold.

Table 4.1.4.7-1. Recovery and distribution of radioactivity after application of 0.15 mg emamectin B_{1a}/kg sandy loam soil and aerobic exposure in the dark (% AR, average of 2 replicates)

Time	CO_2	Extractable	MAB_{1a}	Extraction 1	Extraction 2	Extraction 4 – Fulvic acid	Extraction 4 – Humic acid	Non-extractable - Humin	Total
0 d	n.p.	91.20	**87.77**[&]	91.20	n.p.	n.p.	n.p.	8.93	100.13
2 d	0.15	92.10	84.91	**92.10**	n.p.	n.p.	n.p.	10.04	102.28
4 d	0.17	98.89	87.29	88.06	10.83	n.p.	n.p.	1.03	100.08
7 d	0.27	98.67	84.39	86.91	11.76	n.p.	n.p.	1.92	100.85
14 d	0.48	96.00	75.64	90.69	5.31	n.p.	n.p.	2.75	99.22
21 d	0.69	103.03	77.90	91.08	**11.95**	n.p.	n.p.	3.27	106.99
30 d	0.95	85.62	62.78	75.38	10.24	1.75	**0.24**	2.29	91.53
60 d	3.31	82.42	39.99	72.22	10.20	2.07	0.07	5.62	93.47
100 d	**5.39**	79.56	33.85	68.87	10.69	**6.02**	0.13	**7.85**	98.94

n.p. = not performed; n.d. = not detected greater than twice background;

& For the half-life calculation, the purity of MAB1a (96.5%) was entered as time 0 value since most of the residues in the non-extractable fraction are considered to be parent compound that were not extractable with the neutral extraction 1 but would have been with the organic extraction 2 (cf. extraction on day 4 where organic extraction 2 was applied for the first time

Table 4.1.4.7-1-2. Recovery and distribution of radioactivity after application of 0.15 mg emamectin B_{1b}/kg sandy loam soil and aerobic exposure in the dark (% AR, average of 2 replicates)

Time	CO_2	Extractable	MAB_{1b}	Extraction 1	Extraction 2	Extraction 4 – Fulvic acid	Extraction 4 – Humic acid	Non-extractable - Humin	Total
0 d	n.p.	89.14	84.69[&]	89.14	n.p.	n.p.	n.p.	9.10	98.24
2 d	0.20	92.04	86.99	**92.04**	n.p.	n.p.	n.p.	10.51	102.74
4 d	0.20	91.56	**87.60**	91.56	n.p	n.p.	n.p.	10.32	102.08
7 d	0.33	95.31	82.40	84.55	10.76	n.p.	n.p.	1.80	97.43
14 d	0.50	84.68	69.58	81.32	3.36	n.p.	n.p.	2.35	90.03
21 d	0.99	100.08	75.39	87.33	12.75	n.p.	n.p.	3.08	104.14
30 d	0.95	88.30	66.44	77.88	10.42	1.59	0.09	2.06	92.98
60 d	2.96	81.14	47.47	71.14	10.00	2.77	0.04	3.67	90.57
100 d	**5.82**	79.05	33.59	68.20	**10.85**	**6.12**	**0.11**	**6.71**	97.80

n.p. = not performed; n.d. = not detected greater than twice background;

[&] For the half-life calculation, the purity of MAB_{1b} (97.1%) was entered as time 0 value since most of the residues in the non-extractable fraction are considered to be parent compound that were not extractable with the neutral extraction 1 but would have been with the organic extraction 2 (cf. extraction on day 7 where organic extraction 2 was applied for the first time)

Table 4.1.4.7-3. 2D-TLC quantification of the major and minor (tentatively) identified degradates (% AR, average of 2 replicates)

Time	emamectin benzoate B_{1a}					emamectin benzoate B_{1b}				
[days]	Zones 6/14&	Zones 5/12&&	Zone 7, (FAB_{1a}	Zone 9, (MFB_{1a} aglycone MSB_{1a})	Zone 10 (AB_{1a})	Zones 6/14&	Zones 5/12&&	Zone 7 (FAB_{1a})	Zone 9, (MFB_{1a} aglycone MSB_{1a})	Zone 10 (AB_{1a})
0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2	<LOQ	2.22	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4	2.22	2.19	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
7	3.16	2.40	0.96	1.15	<LOQ	4.42	1.10	1.19	0.88	<LOQ
14	7.36	0.44	1.94	1.81	2.02	5.98	<LOQ	1.32	1.09	1.70
21	7.77	0.90	2.32	1.73	1.30	6.85	2.68	**2.71**	1.97	<LOQ
30	3.57	3.59	**2.44**	0.85	0.78	7.70	0.10	2.70	1.36	<LOQ
60	**10.22**	3.54	2.15	2.14	1.59	9.48	2.71	1.33	**2.13**	**1.11**
100	9.52	**3.91**	1.90	**2.35**	**3.48**	**10.73**	**4.48**	2.26	1.88	0.80

& multicomponent (at least 3 components); && proposed as carboxylic acid of the cleaved macrocycle;

LOQ = limit of quantification.

According to the chromatographic analyses, all kinetic and identification extracts had similar qualitative profiles. The major degradation product for both compounds observed in the 2D-TLC analyses was a 2-zone complex 'zones 6/14' that has been found also in the aerobic aquatic metabolism study (cf. 7.8.3/02) and has been shown by LC/MS to be multicomponent with at least 3 components, containing acid groups in their structures. The sum of this multicomponent reached maximum levels of 10.22% AR on day 60 in the emamectin B_{1a}-treated samples and 10.73% AR on day 100 in the emamectin B_{1b}-treated samples. TLC zone 12, proposed as carboxylic acid product of the cleaved macrocycle, migrated also further up the TLC-plate into zone 5 when re-spotted in a different solvent. The summed zones 5 and 12 peaked at 3.91% AR on day 100 in the emamectin B_{1a} kinetic samples and at 4.48% AR on day 100 in the emamectin B_{1b} identification samples.

Up to 13 other minor TLC zones were seen and each was ≤ 3.48% AR and ≤ 2.71% AR for the two kinetic experiments, respectively. Zone 9 (multicomponent) co-chromatographed with standards MFB_{1a}, aglycone and MSB_{1a}, and zone 7 and 10 co-chromatographed with standards FAB_{1a} and AB_{1a}, respectively. The sum of zone 6 & 14 reached >10% AR at the end of the study. LC/MS showed these zones were multi component (at least 3 components). All four TLC quadrants represent combined multiple trace radiocarbon regions and were ≤ 7.16% AR (MAB_{1a}) and ≤ 6.57% AR (MAB_{1b}).

The author calculated the degradation rate fro the active substance based on the sum of extraction 1 and 2. The average percent total dose from replicates A and B was calculated for each timepoint. Two fitting methods were applied, Origin curve fit (Version 6.0) and Modelmaker version 3.0 with Single First order fit. Both approaches showed a good visual fit.

Table 4.1.4.7-4. DT_{50}-values for emamectin B_{1a}, emamectin B_{1b} and metabolites reported by author

Compound	Soil	DT_{50} [d]	r^2	χ^2	Notes
emamectin benzoate B_{1a}	sandy loam	57.8	0.960	21.4	Origin
emamectin benzoate B_{1b}	sandy loam	63.0	0.959	19.2	

Compound	Soil	DT_{50} [d]	r^2	χ^2	Notes
emamectin benzoate B_{1a}	sandy loam	53.3	0.975	Not reported	Modelmaker
emamectin benzoate B_{1b}	sandy loam	54.6	0.970	Not reported	

Remarks

The data was also evaluated using FOCUS Kinetics guidance. DT_{50}-values of emamectin B_{1a} and emamectin B_{1b} were calculated as reported in table 4.1.4.7-5.

Table 4.1.4.7-5. Recalculated DT_{50}-values for emamectin B_{1a}, emamectin B_{1b} and metabolites.

Compound	Soil	DT_{50} [d]	r^2	χ^2	Visual fit	residuals
emamectin benzoate B_{1a}	sandy loam	63.7	0.964	12.6	MM fit good	Not homogene
emamectin benzoate B_{1b}	sandy loam	71.6	0.969	3.5	Good	Homogene

Although in other soil degradation studies 8a-OH MAB_{1a} was reported to be a major metabolite in soil it was not used as reference substance. Soil microbial biomass at start and end of the study was rather low (99.9-128.4 mg C/kg soil). According to Anderson and Domsch (1989) soils with a biomass within the range of 147-734 mg C/kg soil are considered relevant. Biodegradation might have been underestimated.

The following results are acceptable:

- DT_{50} values (25 °C): 63.7 days for emamectin B_{1a} and 71.6 days for emamectin B_{1b}, recalculated to 20 °C: 95 and 107 days, respectively.

4.1.4.7 Study 8 - Route and rate of degradation in soil - Aerobic degradation

Study reference: Chukwudebe, A. (1994a), STUDY IIA, 7.1.1/004

Detailed study summary and results

Reference/notifier	:	Chukwudebe, A. (1994a)	GLP statement	:	yes
Type of study	:	degradation in soil	Guideline	:	EPA Subdivision N, Section 162-1, 1982
Year of execution	:	1994	Acceptability	:	acceptable
Test substance	:	emamectin benzoate B_{1a}, radiochemical purity 99.8%			

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH	pF	Duration [d]	DT_{50} [d]
emamectin benzoate B_{1a}	sandy loam	aeroob	5	25	2.0	6.6	2.2	366	138.6

Methods

Soil. Sandy loam (Fayette County, Kentucky) was air air dried and 2 mm sieved. The characteristics of the soil are reported in table Table 4.1.4.8-1. Microbial viability of soil was determined prior to the test using plating technique: aerobic bacteria 1.1 x 10^6 CFU/g; actinomycetes: 7.9 x 10^5 CFU/g; fungi: 1.7 x 10^3 CFU/g.

Table 4.1.4.8-1. physico chemical characteristics of the Fayette soil

Parameter	
pH	6.6
Texture Class:	Sandy Loam
% Sand	68
% Silt	23
% Clay	9
Organic Carbon	1.17%
Cation Exchange Capacity	7.5 meqM+/100 g
Field Capacity (at 0.33 bar)	17.1%
Bulk Density (g/cm^3)[1]	1.24

[1] Performed by the University of Kentucky, College of Agriculture, Lexington, Kentucky.

Methods.

Soil was treated with ^{14}C-MAB$_{1a}$, dispersed in ethanol, nominal application rates 0.9 and 5 mg/kg dry soil. Soil was moistened to 75% FC at pF 2.5 and incubated in the dark at 25 ± 1 °C. Approximately every two weeks and at the time of sampling, the flasks were flushed with oxygen via an inlet tube. Organic volatiles and CO_2 were trapped with ethylene glycol and KOH. Duplicate test flasks were sampled at 0, 1, 3, 7 and 14 days and 1, 2, 3, 4, 6, 9, and 12 months.

Analysis.

At each sampling time, the soil samples were dried mixed and then aliquots of the soils were combusted for determination of total radioactive residues.

Soil samples intended for profiling and ^{14}C-MAB$_{1a}$ determination were extracted ammominum acetate (NH_4Ac) in methanol (MeOH), the soil was extracted twice with 180mM NH_4Ac in MeOH:180mM NH_4Ac in H_20 (4:1, v/v). For the samples of day 7 and onwards, the extracted soils were further extracted twice each with 50mL ethyl acetate:ammonium hydroxide (10:1, v/v). One replicate sample of days 14 and 30 was additionally extracted twice with 180mM NH_4Ac in MeOH:180 mM NH_4Ac in H_20 (1:5, v/v) the final extraction step was performed with ethyl acetate:ammonium hydroxide (10:1, v/v). At each step, the volumes of the supernatant were measured and the total radioactivity was quantified by LSC. Post-extraction solids were combusted and the radioactivity measured by LSC. For HPLC analysis, subsamples of the extracts from all extraction steps were combined and concentrated. The extracts of the 2nd replicate of day 0 and day 1 and 3 were analysed directly by HPLC without concentration.

The remainder of all soil samples used for characterisation work were subjected to a similar extraction procedure. For HPLC analyses, subsamples of the extracts from all extraction steps were combined, concentrated under and subjected to HPLC analysis for characterisation of MAB$_{1a}$ and related residues. Additional characterisations were also conducted on purified isolates obtained from these extracts by mixed mode HPLC and RP-HPLC-MS/MS.

Reference substances used: emamectin benzoate B_{1a}, MSB_{1a}, AB_{1a}, 8a -oxo MAB_{1a}, 8a-OH MAB_{1a}, and 8,9-Z-MAB_{1a}. Aglycone was also used as a reference substance for MS characterisation.

Calculations

The half-life of MAB_{1a} was calculated using pseudo first order reaction kinetics using linear regression.

Results

Due to analytical problems encountered in detecting decreasing MAB_{1a} residues from the low soil fortifications (0.09 mg/kg dry soil), only the high dose (5.0 mg/kg dry soil) soil samples were used for the quantification (including half-life determination) and characterisation of MAB_{1a} residues in soil.

Average material balance throughout the exposure phase of the study varied between 94.4 and 105.1% of the initially applied radioactivity (AR).

Distribution of radioactivity is given in Table 4.1.4.8-2. Maximum levels are indicated in bold.

Table 4.1.4.8-2 Recovery and distribution of radioactivity after application of MAB_{1a} to soil and aerobic exposure in the dark (% AR, average of 2 replicates).

Time [d]	CO_2	Extractable	MAB_{1a}	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Non-extractable	Total
0	n.p.	97.1	96.6	n.d.	n.d.	n.d.	0.5	3.6	100.6
1	n.d.	93.2	90.1	1.7	0.7	n.d.	n.d.	5.4	98.6
3	<0.1	93.5	92.7	n.d.	0.9	n.d.	n.d.	6.9	100.5
7	0.2	97.0	83.2	8.8	**3.6**	n.d.	1.4	4.7	101.9
14	0.9	94.6	78.3	12.5	3.4	n.d.	1.0	5.9	101.4
30	2.3	93.7	75.3	4.5	2.5	**1.0**	7.8	9.1	105.1
62	3.1	79.8	51.0	15.9	3.5	n.d.	6.8	15.0	97.9
90	5.1	73.8	46.8	15.0	3.6	n.d.	8.4	18.1	97.3
122	8.3	68.7	38.7	14.4	2.9	0.9	**10.8**	19.9	96.9
182	10.2	65.0	35.7	16.3	0.6	n.d.	8.1	22.0	97.5
274	14.3	55.9	30.9	18.5	n.d.	n.d.	6.6	**27.4**	97.7
366	**16.3**	52.7	27.1	**21.4**	1.1	n.d.	3.2	25.2	94.4

n.p. = not performed; n.d. = not detected greater than twice background

In addition to parent MAB_{1a} (retention time 34-38.5 min), four unknown fractions were eluted prior to the parent (fractions 1-4). Approximate retention times of unknown fractions were: 3.5-7.5 minutes, 8.5-12.5 minutes, 16.5-26.6 minutes, 28.5-35.0 minutes for fractions 1 to 4 respectively.

RP-HPLC/MS/MS revealed one individual degradate, i.e. 8a-OH-MAB_{1a}, which is slightly more polar than parent MAB_{1a}, and a complex polar fraction eluting before the monosaccharide (MSB_{1a}) of MAB_{1a}, that consists of at least 18 different polar components. The major residue component is the polar fraction, cumulatively comprising a maximum of 18.6% AR at the 1-month incubation interval. The polar fraction was resolved into different components using mixed mode HPLC, with the major components constituting at least 1% but less than 7% AR. However, there is some evidence that even the biggest component is a mixture consisting of several co-eluting, but discrete residues.

The maximum level for 8a-OH-MAB_{1a} was found at the 1-month sampling comprising ca. 8.3% AR but did not exceed two times 5%. In addition, the possible presence of 8a-oxo-MAB_{1a} residues at levels much lower than that of 8a-OH-MAB_{1a} was based on similarities in molecular mass between the soil isolate and the 8a-oxo-MAB_{1a} reference standard. However, daughter ion spectra were not obtained for this unknown soil residue and therefore a structural assignment to either 8a-oxo-MAB_{1a} or another minor MAB_{1a} derivative of the same molecular weight cannot be definitely made.

Half-life of emamectin benzoate B_{1a} reported by the author is 193.4 days.

Remarks

The DT_{50} was recalculated according to FOCUS kinetics recommendations using ModelMaker 4.0.

Table 4.1.4.8-3. Recalculated DT_{50}-values for emamectin B_{1a},

Compound	Fit	Soil	DT_{50} [d]	r^2	χ^2	Visual fit	residuals
emamectin benzoate B_{1a}	SFO	sandy loam	138.6	0.905	10.1	Not very good	acceptable
	FOMC	Sandy loam	92.1	0.985	4.2	good	acceptable

Soil metabolites MFB_{1a} and N-nitroso MAB_{1a} were not used as reference substances although they were used in other soil metabolism studies. Soil history is not reported.

As the SFO fit is considered acceptable enough with no major increase in fitting quality using FOMC kinetics, the SFO value is used for modelling.

- DT_{50} for emamectin benzoate B1a in sandy loam soil (aerobic, 25 °C): 138.6 days, recalculated to 20 °C: 207 days

4.1.4.8 Study 9 - Route and rate of degradation in soil - Anaerobic degradation

Study reference: Chukwudebe, A. (1995b), STUDY IIA, 7.1.2/001

Detailed study summary and results

Reference/notifier	:	Chukwudebe, A. (1995b)	GLP statement	:	yes
Type of study	:	anaerobic degradation in soil	Guideline	:	EPA Subdivision N, Section 162-1, 1982
Year of execution	:	1991-1992	Acceptability	:	not acceptable
Test substance	:	[^{14}C]-emamectin benzoate B_{1a} (MAB_{1a}) radiochemical purity 99.6%			

Substance	Soil type	Condition	Dose [mg/kg]	T [° C]	OM [%]	pH	pF	Duration [d]	DT_{50} [d]
emamectin B_{1a}	sandy loam	anaeroob	5.0	24.0-26.0	2.3	7.0	2.5	60	-

Materials and methods:

Soil. Sandy loam (Fayette County, Kentucky) was air air dried and 2 mm sieved. The characteristics of the soil are reported in Table 4.1.4.9-1. Microbial viability of soil was determined prior to the test using plating technique: aerobic bacteria 1.1 x 10^6 CFU/g; actinomycetes: 7.9 x 10^5 CFU/g; fungi: 1.7 x 10^3 CFU/g.

Table 4.1.4.9-1 . physico chemical characteristics of the Fayette sandy loam soil

Parameter	
pH	7.0
Texture Class:	Sandy Loam
% Sand	71
% Silt	21
% Clay	8
Organic Carbon	1.37%
Cation Exchange Capacity	8.2 meq/100 g
Field Capacity (at 0.33 bar)	14.2%
Bulk Density (g/cm^3)[2]	1.24

[1] PTRL Soil No.: A-15

[2] Performed by the University of Kentucky, Department of Agriculture, Lexington, Kentucky. Bulk density was determined using the core method as described in "Methods of Soil Analysis" published by the American Society of Agronomy, Inc., Agronomy No. 9, 1965.

Methods

A methanol solution of ^{14}C-MAB_{1a} was applied evenly to the soil, nominal application rate 5.0 mg/kg dry soil. Soil was moistened to 75% FC at 0.33 bar and aged under aerobic conditions for 30 days in a closed system in the dark at 25 °C. Anaerobic conditions were then introduced by flushing the flasks with nitrogen. Flasks were maintained under nitrogen for another 60 days. Organic volatiles and CO_2 were trapped with ethylene glycol and KOH. Duplicate samples were taken on days 0, 30, 59 and 90.

Analysis.

At each sampling time, aliquots of the soils were combusted for determination of total radioactive residues. Soil samples intended for profiling and ^{14}C-MAB_{1a} determination were extracted with 180mM ammominum acetate in MeOH. The soil was extracted twice with 180mM NH4Ac in MeOH:180mM NH4Ac in H_20 (4:1, v/v). For all soil samples except one replicate of day 0, the extracted soils were further extracted three times each with ethyl acetate:ammonium hydroxide (10:1, v/v). At each step, the volumes of the supernatant were measured and the total radioactivity was quantified by LSC. Post-extraction solids were combusted and the radioactivity measured by LSC. For HPLC analysis, subsamples of the extracts from all extraction steps were combined, concentrated under nitrogen and stored at ca. –20°C until HPLC analysis.

The remainder of all soil samples was subjected to a similar procedure as described before. The next step was a repeated extraction with 30mL MeOH:H_20 (1:1, v/v). Finally the soils were extracted five times each with ethyl acetate:ammonium hydroxide (10:1, v/v). At each step, the volumes of the supernatant were measured and the total radioactivity was quantified by LSC. For HPLC analyses, subsamples of the extracts from all extraction steps were combined, concentrated and subjected to HPLC analysis for characterisation of MAB_{1a} and related residues. Additional characterisation was also conducted on polar fraction isolates, obtained from a selected soil (i.e., day 59) extract.

Reference substances used: MAB_{1a}, monosaccharide of MAB_{1a} (MSB_{1a}), 8a-OH-MAB_1, and 8a-oxo-MAB_1.

Calculations

The half-life of MAB_{1a} was calculated using pseudo first-order reaction kinetics with linear regression.

Results

Average material balance throughout the exposure phase of the study varied between 91.4 and 99.8% of AR).

Distribution of radioactivity given in Table 4.1.4.9-2.

Table 4.1.4.9-2 Recovery and distribution of radioactivity after application of MAB_{1a} to soil and aerobic/anaerobic exposure in the dark (% AR, average of 2 replicates).

Time	CO_2	Extractable	MAB_{1a}[&]	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Non-extractable	Total
Aerobe exposure									
0 d	n.p.	94.8	88.6	n.d.	n.d.	3.8	2.4	2.2	97.0
30 d	0.6	80.8	66.8	4.9	1.7	7.5	n.d.	10.0	91.4
Anaerobic exposure									
59 d	2.0	81.2	63.4	6.3	1.4	10.0	n.d.	16.7	99.8
90d	3.1	75.0	60.6	6.7	1.2	6.6	n.d.	15.6	93.6

n.p. = not performed; n.d. = not detected

In addition to parent MAB_{1a} (retention time 34-38.5 min), four unknown fractions were eluted prior to the parent. The four unknown fractions were eluted prior to the parent at 4.0-6,5 minutes (Fraction 1), 9.0 minutes (Fraction 2), at 29.0-33.5 minutes (Fraction 3), and at 40.0-40.5 minutes (Fraction 4).

More extensive analysis of soil extracts using up to three different HPLC methods revealed two individual degradates, i.e. 8a-OH-MAB_{1a} and 8a-oxo-MAB_{1a}, which are slightly more polar than parent MAB_{1a}, and a complex polar fraction eluting before the monosaccharide (MSB_{1a}) of MAB_{1a} that consists of at least 8 different polar components. The major residue component is the polar fraction, cumulatively comprising a maximum of about 13 and 11% AR at the 59 and 90-day incubation interval with none of the individual components constituting ≥ 4% AR. The maximum level for 8a-OH-MAB_{1a} and 8a-oxo-MAB_{1a} was found at the 59-day sampling, comprising (by RP-HPLC only) 8.0 and 3.4% AR, respectively. Particularly the level for 8a-OH-MAB_{1a}, however, could not be confirmed by other HPLC methods (NP-HPLC, RP-HPLC with different selectivity). Accordingly, the 8a-OH-MAB_{1a} and 8a-oxo-MAB_{1a} residues at various samplings averaged using 3 HPLC methods were either similar to, or less than 4.4% AR. The levels of 8a-OH-MAB_{1a}, 8a-oxo-MAB_{1a} and complex polar fraction at 30 days, i.e., aerobic incubation, were not significantly less compared with the 59- and 90-day intervals, i.e. anaerobic incubation, indicating that MAB_{1a} is more efficiently degraded under aerobic than under anaerobic conditions.

Reported cumulative DT_{50} (over all sampling dates) of emamectin benzoate B_{1a} reported by the author is 174.2 days. Reported anaerobic DT_{50} of emamectin benzoate B_{1a} was 427.4 days.

Reported anaerobic DT_{50} value is based on three sampling dates only and is considered not reliable by RMS.

Remarks

MFB_{1a}, FAB_{1a}, aglycone, AB_{1a}, 8,9-Z-MAB_{1a} and N-nitroso MAB_{1a} were not used as reference substances. Soil history is not reported. No $DT_{50,\ anaerobic}$ could be derived.

4.1.4.9 Study 10 - Photochemical degradation in water

Study reference: Ballantine, L.G. (1994), STUDY IIA, 7.6/01

Detailed study summary and results

Reference/notifier	:	Ballantine, L.G. (1994)	GLP statement	:	yes
Type of study	:	photolysis in water	Guideline	:	US-EPA Subdivision N, 161 – 2, 1982
Year of execution	:	1992 – 1993	Acceptability	:	acceptable
Test substance	:	[3,7,11,13,23-^{14}C]4"-epimethylamino-4"-deoxyavermectin B_{1a} benzoate; batch nr. L-683,825-003E006; radiochemical purity 96.7%			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [h]	Quantum yield	Transformation at end [%]	DT_{50} photo [h]
^{14}C-MAB_{1a}	phosphate buffer with ethanol	25	7	Xenon	> 290	142	-	62 -65	6.3-8.5
^{14}C-MAB_{1a}	phosphate buffer with acetonitrile	25	7	Xenon	> 290	737	-	37 - 56	31.8-64.5
^{14}C-MAB_{1a}	phosphate buffer with acetone	25	7	Xenon	> 290	24	-	55 - 73	0.5-1.0

Description

Methods. Test solutions were prepared in 0.01 M phosphate buffer with 1% ethanol. Samples were irradiated in a flow-through system. Volatiles were trapped in a set of three traps, in 0.1 N sulphuric acid, in ethylene glycol and 2 N NaOH. The exposure time was 142 and 189 hours for the 5 and 10 mg/L samples, respectively. Light intensity was monitored, sterility of buffers confirmed. Based on the results of the flow-through system, showing that neither ^{14}C-volatiles nor ^{14}C-CO_2 were produced. MAB_{1a} solutions in 0.01 M phosphate buffer, at pH 7, containing either 1% acetonitrile or 1% acetone as a cosolvent, were irradiated in sealed ampules (closed system). The exposure time was 737 hours in the test with acetonitrile and 24 hours in the test with acetone. Besides the ampules used for the rate experiments, greater borosilicate glass ampules (10 mL) filled with 7 mL of acetonitrile and acetone blended 30 mg/L test solution were set up under identical conditions for the purpose of characterisation of potential degradates. In all experiments, irradiation was performed with a Xenon lamp with UV-filter (λ > 290 nm), 12:12 hours L:D. Incubation for 37.5 days.

Chemical analysis. Duplicate samples were removed periodically over the exposure period, and aliquots were analysed by LSC for concentration measurement and by HPLC for preliminary characterisation of isolated fractions. Bulk aqueous solutions of ^{14}C-MAB_{1a} (30 mg/L) containing either acetone or acetonitrile co-solvents were subjected to more sophisticated HPLC analysis for further photodegradate identification. Based on the findings of this initial analysis, the sample showing the most extensive degradation was chosen for fractionation using reverse phase semi-preparative HPLC. Then the individual residues were isolated by normal phase HPLC and identification work was conducted using various reference standards.

Calculations. $DT_{50,\ photolysis}$ of MAB_{1a} was estimated by linear first order kinetics.

Results

The spectral energy distribution of the light source was measured to be less than that of natural sunlight at midday on July 15, 1991 at the test site (89° W longitude, 43° N latitude). Between 260 and 400 nm distribution was similar but between 400 and 800 nm the intensity was consistently circa 50 watt/m^2 less.

Mass balance: Recoveries of applied radioactivity at each sampling interval ranged from 80.7 to 103.5% (mean: 92.8 ± 6.8%) in the irradiated samples and from 74.7 to 103.5% (mean: 92.9 ±7.9%) in the dark controls.

Flow-through system with ethanol as co-solvent: in the irradiated samples of the 5 and 10 mg/L treatments the concentration of MAB_{1a} decreased from 76.6 and 79.9% at 0 hours (values so low probably due to mixing or sampling error) to 35.5 and 37.6% at 142 and 189 hours, respectively. Volatile components were not detected in significant quantities in the trapping solutions (<2% of the applied radioactivity, AR). In the

dark control, no degradation occurred as indicated by MAB1a concentration of 97.8% at 142 hours. The findings are summarised in Table 4.1.4.10-1.

Table 4.1.4.10-1: Photolysis of MAB_{1a} in aqueous buffer pH 7 with 1% ethanol co-solvent in a flow-through system (% AR, average of 2 replicates)

Time (hours)	MAB_{1a} concentration: 5 mg/L						Time (hours)	MAB_{1a} concentration: 10 mg/L		
	Irradiated samples			Dark control				Irradiated samples		
	MAB_{1a}	P1	P4	MAB_{1a}	P1			MAB_{1a}	P1	P4
0[&]	76.6	<0.1	<0.1	76.6	<0.1		0*	79.9	<0.1	<0.1
24	75.1	5.5	5.9	94.9	<0.1		21	74.4	5.4	6.6
48	53.9	19.2	5.8	95.5	<0.1		64	66.1	10.0	7.9
94	68.9	21.9	7.2	103.4	9.4		140	61.4	14.3	7.3
142	35.5	35.0	3.3	97.8	1.8		189	37.6	39.8	3.9

[&] Recovery in the 0 hour samples was lower than expected, due to possible mixing or sampling error

Sealed ampules and acetonitrile as co-solvent: in the irradiated samples of the 10 and 30 mg/L treatment the concentration of MAB_{1a} decreased from 91.7 and 93.1% at 0 hours to 62.7 and 43.9% at 737 hours, respectively. In the dark control, no degradation occurred as indicated by MAB_{1a} concentrations of 90.3 and 92.8 mg/L at 737 hours, respectively. The findings are summarised in Table 4.1.4.10-2.

Table 4.1.4.10-2. Photolysis of MAB_{1a} in aqueous buffer pH 7 with 1% acetonitrile co-solvent in a closed system (% AR, average of 2 replicates).

Time (hours)	MAB_{1a} concentration: 10 mg/L						MAB_{1a} concentration: 30 mg/L				
	Irradiated samples			Dark control			Irradiated samples			Dark control	
	MAB_{1a}	P1	P4	MAB_{1a}	P1		MAB_{1a}	P1	P4	MAB_{1a}	P1
0	91.7	0.0	0.0	91.7	0.0		93.1	0.0	0.0	93.1	0.0
24	85.9	0.9	3.3	n.s.	n.s.		90.4	1.3	3.5	n.s.	n.s.
49	85.4	1.6	5.9	n.s.	n.s.		82.1	1.5	4.9	n.s.	n.s.
87	80.8	1.9	4.3	n.s.	n.s.		83.1	2.0	6.6	n.s.	n.s.
168	75.8	3.2	8.0	95.0	0.0		77.1	3.2	7.1	94.0	0.0
216	74.7	4.3	7.5	n.s.	n.s.		78.0	5.0	7.5	n.s.	n.s.
258	77.6	6.8	7.3	n.s.	n.s.		70.1	4.5	7.5	n.s.	n.s.
330	75.2	4.8	7.2	93.8	0.0		72.0	7.4	8.8	92.6	0.0
382	74.1	5.5	8.2	n.s.	n.s.		70.4	8.5	8.3	n.s.	n.s.
425	64.6	10.8	8.7	n.s.	n.s.		64.5	11.4	8.4	n.s.	n.s.
496	70.4	8.6	8.8	94.0	0.0		63.8	14.8	7.9	94.3	0.0
598	68.2	6.8	8.8	95.2	1.7		50.5	20.6	6.8	n.s.	n.s.
737	62.7	12.9	8.9	90.3	0.8		43.9	27.3	6.7	92.8	0.0

n.s. = not sampled

Sealed ampules and acetone as co-solvent: in the irradiated samples of the 12 and 32 mg/L treatment the concentration of MAB_{1a} decreased from 92.1 and 87.1% at 0 hours to 26.7 and 44.6 at 24 hours, respectively. No dark controls were used in this sub-test. The findings are summarised in Table 4.1.4.10-3.

Table 4.1.4.10-3: Photolysis of MAB_{1a} in aqueous buffer pH 7 with 1% acetone co-solvent in a closed system (% AR, average of 2 replicates).

Time	MAB_{1a} concentration: 12 mg/L						MAB_{1a} concentration: 32 mg/L				
(hours)	MAB_{1a}	P1	P2	P3	P4		MAB_{1a}	P1	P2	P3	P4
0	92.1	<0.1	<0.1	<0.1	<0.1		87.1	<0.1	<0.1	<0.1	<0.1
3	76.1	3.1	2.1	4.9	4.5		84.5	1.6	1.3	2.1	5.5
7.5	58.7	8.1	6.1	8.5	5.9		74.1	4.0	2.7	3.5	10.8
15	38.1	21.0	13.8	12.9	2.4		50.8	9.0	6.3	9.0	10.9
24	26.7	18.4	16.2	12.8	1.8		44.6	13.4	8.9	9.7	8.8

The experimental half-life (DT_{50}) are summarised in Table 4.1.4.10-4.

Table 4.1.4.10-4. Photolytic half-life of MAB_{1a} in aqueous buffer pH 7.

	1% ethanol co-solvent		1% acetonitrile co-solvent		1% acetone co-solvent	
	5 mg MAB_{1a}/L	10 mg MAB_{1a}/L	10 mg MAB_{1a}/L	30 mg MAB_{1a}/L	12 mg MAB_{1a}/L	32 mg MAB_{1a}/L
Half-life (days)	6.3	8.5	64.5	31.8	0.5	1.0

Photodegradates

For the characterisation of potential photodegradates of MAB_{1a}, a 24-hour acetone sensitised sample was chosen because it was the most extensively degraded of the samples. A total of 16 individual residues (Table 4.1.4.10-5) from this sample were characterised with six of these (MAB_{1a}, AB_{1a}, 8a-OH MA, 8a-oxo MA, 8,9-Z MA, and the di-epoxide) being identified.

The concentration of these residues ranged from 36.21% (MAB_{1a}) to 0.52% (AB_{1a}) with no residue besides parent comprising more than approximately 7% of the total radioactivity in this sample. The concentration of the ten residue components (P3a, P3b, P3b-1, P3c, P3d, P1a, P1b, P1c, P1d, P1e), which were characterised but not identified, ranged from 6.55% (P3b) to 0.19% (P1d). In all, these 16 components accounted for 63.28% of the radioactivity in the 24-hour sensitised sample. The remaining 36.72% of the radioactivity was classified as not identified and did not include any distinct peaks.

Table 4.1.4.10-5. Summary of residue component percentages in the 24-hour acetone sensitised aqueous sample

Component	MAB_{1a}	8,9-Z MAB_{1a}	Di-epoxide	P3a	P3d	P3c	8a-oxo MA	8a-OH MA
Prelim. comp.	MAB_{1a} + P4	P4	P2 + P3	P3 + P2	P2	P3	P3	P3
% of total radioactivity in sample	36.21	6.57	1.12	1.17	0.36	2.44	2.35	0.97

Component	P3b	P3b-1	AB_{1a}	P1a	P1b	P1c	P1d	P1e	Not identified
Prelim. comp.	P3	P3	P3	P1	P1	P1	P1	P1	--
% of total radioactivity in	6.55	1.71	0.52	2.44	0.25	0.30	0.19	0.14	36.72

Component	P3b	P3b-1	AB_{1a}	P1a	P1b	P1c	P1d	P1e	Not identified
sample									

prelim. comp. = component according to preliminary HPLC analysis

HPLC analysis of additional acetone sensitised samples indicated that the two major initial photodegradation pathways were 8,9 photo-isomerization and epoxidation, accompanied by an increase in the "polar" P1 residues with increasing time. In non-sensitized samples, only the 8,9-Z photo-isomer and the polar P1 type residues were seen.

Direct photolysis appears to be no significant degradation path of MAB_{1a} in aqueous solution with 1% acetonitrile as a co-solvent (half-life 32 – 65 days). A photosensitizer (acetone) or a radical hydrogen donor (ethanol) cause a faster degradation (0.5 – 8.5 days).

The photolytic degradation route under sensitised conditions was complex with up to 15 distinguishable components after 24 hours irradiation none of which represented ≥10% AR. The major residues at 24 hours were parent MAB_{1a} (36.2%), 8,9-Z isomer of MAB_{1a} (6.6% AR), and P3b (6.6% AR), while the remaining components were ≤2.4% AR.

Remarks

Recalculation of $DT_{50,photolysis}$ for MAB_{1a} gives similar results. Recalculation to DT_{50} under natural conditions is not possible, as the light intensity in the study was not measured.

The following results as calculated by the author are acceptable:

- Direct photolysis of MAB_{1a} in aqueous solution (with 1% acetonitrile as co-solvent) exists but is slow (half-life 32 – 65 days).
- Acetone or ethanol causes a faster degradation (0.5 – 8.5 days).
- The photolytic degradation route under sensitised conditions was complex with up to 15 distinguishable components after 24 hours irradiation none of which represented ≥ 10% AR. The major residues at 24 hours were parent MAB_{1a} (36.2%), 8,9-Z isomer of MAB_{1a} (6.6% AR), and P3b (6.6% AR), while the remaining components were ≤ 2.4% AR. ll

4.1.4.10 Study 11 - Photochemical degradation in water

Study reference: Mushtaq, M. (1995), STUDY IIA, 7.6/02

Detailed study summary and results

Reference/notifier	:	Mushtaq, M. (1995)	GLP statement	:	yes
Type of study	:	photodegradation in water	Guideline	:	EPA Subdivision N, Section 161-2, 1982;
Year of execution	:	1993-1995	Acceptability	:	acceptable
Test substance	:	[3,7,11,13,23-^{14}C]-emamectin B_{1a} benzoate (^{14}C-MAB_{1a}), L-683,825-003E003, radiochemical purity 98 %; MAB1a, L-656,748			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [h]	Transformation at end [%]	DT_{50} photo [d]
[^{14}C]- MAB_{1a}	phosphate buffer	25 ±1	6.9-7.0	natural sunlight	> 290	30	53.1	22.4
	sensitised phosphate buffer		7.0	natural sunlight	> 290	30	99.1	1.4
	natural pond water		7.4-8.9	natural sunlight	> 290	30	95.8	6.9

Description

Aqueous photodegradation of emamectin benzoate (test substance: ^{14}C-MAB_{1a}) in three experiments:

(i) 0.1M phosphate buffer pH 7.0;

(ii) 0.1M phosphate buffer pH 7.0 plus 1% acetone as sensitizer;

(iii) natural pond water from a pond at the test site (15.6 mg Ca^{2+}/L, 7.8 mg Mg^{2+}/L, 15.4 Na^{+}/L, 45 mg/L total organic carbon, 76 mg/L total hardness, 267 µmhos/cm specific conductance, pH 7.53).

The nominal initial concentration was approximately 1 mg/L. All the sample tubes were exposed to natural sunlight (ca. 10 hours of light cycle per day) with the average light intensity varying with weather conditions. Light exposed tubes in three replicates. Dark controls in one replicate. Duplicate test tubes from each treatment were removed after sunlight exposure at days 1, 3, 7, 14, 21 and 30, and in addition at days 2, 5, and 10 for the sensitised buffer. All sample tubes were stored in a freezer until the day of analysis.

Conditions: natural sunlight at the test site (latitude ca. 40°N) in the fall season.

Analysis: Analysis by LSC for concentration measurement. Two extractions with ethyl acetate and extracts were combined. Radioactivity was determined in both the organic and aqueous phases by LSC. A 3 mL aliquot of the combined organic extract was mixed with emamectin standard solution, dried under nitrogen and reconstituted in methanol. Aliquots of both the reconstituted organic extract and the extracted aqueous phase were then analysed by reversed-phase HPLC. Fractions of the HPLC runs were collected and used for further identification work of potential degradates via HPLC using reference standards (8a-OH MAB_{1a}, AB_{1a}, MAB_{1a}, 8,9-Z-MAB_{1a}, MAB_{1a}-diepoxide, 8a-OH MAB_{1a}).

Calculations: Based on the measured MAB_{1a} concentration in the test solutions at the different intervals, the half-life of MAB_{1a} was calculated on basis of linear first-order kinetics. UV-visible absorbance of two MAB_{1a} solutions in methanol was determined at wavelengths from 297.5 to 800 nm and converted to molar absorbance. These molar absorbance values in combination with solar irradiance data for summer, fall and winter as published by US EPA and the determined half-life of MAB_{1a} in fall were used to estimate half-lives of MAB_{1a} in the different test media also in summer and winter.

Results

The average light intensity varied with weather conditions. The sunny days ($6.0 \times 10^{-6} – 1.1 \times 10^{-5}$ W/cm^2 nm) had a 3-5 fold higher average light intensity at 450 nm in comparison to overcast days ($1.7 \times 10^{-7} – 2.1 \times 10^{-6}$ W/cm^2 nm). The temperature dropped below 25°C several times due to the natural changes in temperature particularly during the night (e.g. 8 – 12°C day 16 to 17).

Mass balance: The recoveries of the total sample radioactivity in the aqueous solution before extraction were on average around 65% but ranged between 50 to 90% with no difference between the light exposed and dark specimens. After extraction with ethyl acetate rinsate of the test tubes, the combined recoveries of the radioactivity in the test solution aliquots, and in the aqueous and organic phase after extraction were ≥ 90% with a range between 70 to 120%. The difference between recoveries before and after extraction was due to adsorption of residues to the quartz tube surface, which however was demonstrated to have no impact on the aqueous photolytic process.

The distribution of the radioactivity between organic (extractable residues) and aqueous (non-extractable residues) phases of the dark controls and zero time light exposed aqueous specimens was ca. 99% : 1%. In the light exposed aqueous specimens, the extractable residues decreased with sunlight exposure time (down to 43 to 84% by day 30 for sensitized and normal buffer, respectively) and a concurrent increase in the non-

extractable residues (up to 16 to 57% by day 30 for normal buffer and sensitized buffer, respectively) was observed (see Table 4.1.4.11-1).

MAB_{1a} was photodegraded between day 0 and 30 by approximately 63 and 96% in the buffer and natural water samples, respectively, whilst in the sensitised buffer MAB_{1a} was almost completely degraded already by day 14. The findings are summarised in Table 4.1.4.11-2.

Table 4.1.4.11-1. Photolysis of MAB_{1a} benzoate in buffer pH 7, natural pond water and sensitised buffer pH 7 (with 1% acetone) exposed to natural sunlight in fall (average % of total residues). In bold the degradates >10% of AR

Sampling day	**Extractable residues**						**Non-extract. residues[&]**
	MAB_{1a}	**MAB_{1a}-10,11-14,15-diepoxide**	**8,9-Z-MAB_{1a}**	**Polar residues[&]**	**Undefined residues**	**Total extractable residues**	**(aqueous phase)**
Buffer pH 7							
0	95.19	0.69	0.80	0.36	2.80	99.83	0.17
1	86.45	1.25	6.40	1.31	4.24	99.64	0.36
3	75.48	1.50	10.95	4.48	6.08	98.50	1.50
7	64.46	2.05	**12.28**	8.24	8.98	96.02	3.98
14	51.89	2.34	10.97	14.58	11.54	91.32	8.68
21	42.25	2.68	10.55	17.88	14.67	88.03	11.97
30	36.87	2.80	9.00	21.03	14.08	83.78	16.22
Natural pond water							
0	95.24	0.74	1.02	0.66	2.00	99.66	0.33
1	68.57	1.25	**17.10**	3.25	7.35	97.53	2.47
3	49.35	2.56	15.89	10.39	13.07	91.26	8.74
7	31.97	3.35	11.18	18.85	14.39	79.73	20.27
14	22.83	3.47	7.90	23.90	14.74	72.84	27.16
21	7.47	3.03	2.83	33.40	8.87	55.60	44.40
30	4.23	2.64	2.05	36.50	7.14	52.56	47.44
Sensitised buffer (buffer pH 7 + 1% acetone)							
0	96.03	0.79	1.14	0.32	1.66	99.94	0.06
1	54.25	3.73	8.40	9.14	20.63	96.14	3.85
2	36.90	8.60	4.95	14.88	26.77	92.11	7.89
3	12.76	16.46	2.2.1	23.73	29.28	84.45	15.55
5	11.13	17.28	1.84	26.26	23.56	80.07	19.94
7	8.26	**18.28**	1.27	26.61	20.55	74.96	25.04
10	6.76	17.02	1.28	31.74	13.17	69.98	30.03
14	2.53	12.86	1.05	36.69	8.82	61.95	38.05
21	1.18	7.49	0.72	38.51	4.51	52.41	47.59
30	0.85	2.90	0.72	36.81	2.22	43.50	56.60

[&] Comprising several very polar residues of unknown identity, each in small quantities

The major degradate in the light exposed buffer and natural water samples was the 8,9-Z-isomer of MAB_{1a} that was found at maximum amounts of 12.3% (day 7) and 17.1% (day 1), respectively. MAB_{1a}-10,11-

14,15-diepoxide was identified also in both systems, however, at amounts not exceeding 2.8% and 3.5%, respectively. Both degradates were found also in the sensitised buffer samples with MAB_{1a}-10,11-14,15-diepoxide occurring at a greater amount (18.3% at day 7) than the 8,9-Z-isomer of MAB_{1a} (8.4% at day 1). In addition to the two identified compounds, the HPLC analyses of the aqueous phase and of the polar HPLC regions (0 – 20 minutes) from organic extracts indicated that these not-identified residues were a mixture of several potential polar degradates, each in small quantity.

The rate of photolytic degradation of MAB_{1a} under the experimental conditions (fall, latitude ca. 40°N) was calculated applying a first-order reaction kinetics model (linear regression) to the measured MAB_{1a} residue values. The half-life values (DT_{50}) for fall are summarised in Table 4.1.4.11-2.

Table 4.1.4.11-2. Photolytic half-life (in days) of MAB_{1a} in aqueous media at latitude ca. 40°N

Season	$\sum$ ελ x Lλ 297.5 – 800 nm	Photodegradation rate in comparison to fall	Buffer pH 7	Natural pond water	Buffer pH 7 + 1% acetone
Fall[1]	15.7598	1	22.4	6.9	1.4
Summer[2]	30.6079	1.9421	11.5	3.6	0.7
Winter[2]	9.9774	0.6331	35.4	10.9	2.2

[1] calculated based on actual experimental data
[2] estimated from fall data using EPA method

8,9-Z-MAB_{1a} was the major degradation product in buffer (12.3%) and natural pond water (17.1%). In sensitised buffer, MAB_{1a}-10,11-14,15-diepoxide was identified as the major degradate (18.3%), followed by 8,9-Z-MAB_{1a} (8.4%). The amount of polar degradates increased significantly with light exposure time in all treatments indicating that they are potential end products in aqueous photolysis. Reverse-phase HPLC analyses indicated that those residues were a mixture of many degradates, each in small quantity, but identification of individual components was not achieved.

Remarks

The metabolite 8a-OH MAB_{1a} was present at <5% but was not indicated in table 4.1.4.11-1. Recalculated DT_{50} values under study conditions were 19.8 days (r^2 0.96) for the buffer, 1.3 days (r^2 0.98) for the sensitized buffer and 5.2 days (r^2 0.95) for the natural pond water. As these half-life values were very similar to the DT_{50} values calculated by the author, the DT_{50} values of the author were acceptable. MAB_{1a}-10,11-14,15-diepoxide and 8,9-Z-MAB_{1a} were identified as major metabolites in (sensitised) buffer and 8,9-Z-MAB_{1a} was identified as the major metabolite in natural pond water.

4.1.4.11 Study 12 - Photochemical degradation in water

Study reference: Phaff (2005), STUDY IIA, 7.6/03

Detailed study summary and results

Reference/notifier	:	Phaff (2005)	GLP statement	:	yes
Type of study	:	photodegradation in water, determination of quantum yield	Guideline	:	OECD draft, August 2000; JMAFF Agchem Test Guidelines 12 Nohsan N.8147, 24.11.2000 (revised 26.06.2001); EPA Subdivision N, Section 161-2, 1982
Year of execution	:	2004-2005	Acceptability	:	acceptable
Test substance	:	[23-^{14}C]-emamectin B_{1a} benzoate, WFH-XI-3, radiochemical purity 99 %			

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [h]	Quantum yield	Transformation at end [%]	DT_{50} photo [d]
[^{14}C]-emamectin B_{1a} benzoate	phosphate buffer	25.3 ± 0.3	6.99-7.06	simulated sunlight	> 290	24	0.0144	55.4	0.9

Description

Aliquots of 50 mL buffer solution were transferred to sterilised glass vessels and 0.5 mL application solution was added and thoroughly mixed, resulting in a initial concentration of 0.91 mg ^{14}C-MAB_{1a}/mL. Additionally, 50 mL of actinometer solution were transferred to a test vessel to determine the number of photons entering the test solution. Sterility confirmed at start of experiment. The vessels were covered with sterilised quartz glass plates (volatiles were not collected since in pre-tests no volatile radioactivity was detected) and placed in a Suntest CPS apparatus (Heraeus, D) fitted with a Xenon arc light source and an UV filter with a 290 nm cut-off. The test vessels containing ^{14}C-MAB_{1a} were irradiated continuously for 24 hours. Duplicate samples of the MAB_{1a} treatments were taken after 0, 1.5, 3, 5, 6.5, 10 and 24 hours. The actinometer solution was irradiated for only 100 minutes. Aliquots (0.2 mL) of the actinometer solution were taken every 20 minutes and subjected to HPLC analysis. The test solutions were exposed to a mean light intensity in the wavelength range of 300 to 400 nm of 47.9 W/m^2.

In addition to the irradiated test vessels, duplicate test vessels containing 15 mL buffer solution and 0.15 mL application solution were incubated under identical conditions but in the dark. Since emamectin is known to be stable to hydrolysis at pH 7, duplicate dark control samples were taken only at 24 hours.

Chemical analysis. At the end of the exposure interval aliquots of the test solution then were subjected to HPLC analysis. Two-dimensional TLC was used as an additional method to verify the HPLC findings. For HPLC the LOD was 0.001 mg parent equivalent/L and the LOQ was 0.003 mg parent equivalent/L.

Calculations. Based on the measured MAB1a concentration in the test solution at the different intervals, the half-life was calculated on basis of linear first-order kinetics. The environmental half-life at the surface of pure water at different northern latitudes and in the River Rhine system was calculated using the computer program GCSOLAR (EPA, v. 1.20, 1999) taking into account the quantum yield value and the absorption data of MAB_{1a} in methanol.

Results

Light intensity was on average 47.9 W/m^2, corresponding value of summer midday sunlight at 50 °N is 50.4 w/m^2, ratio of intensity 0.95. Irradiation (12 h) corresponded to 0.77 days at 30 - 50 °N.

Mass balance: Recoveries of applied radioactivity at each sampling interval ranged from 98.1 to 103.2% (mean: 100.8 ±1.7%) in the irradiated samples and from 100.5 to 101.0% (mean: 100.8 ±0.2%) in the dark control.

In the irradiated samples, the concentration of MAB_{1a} decreased steadily from 99.3% at 0 hours to 44.6% at 24 hours (cf. Table 4.1.4.12-1). Besides the parent compound up to 18 fractions were detected in the HPLC chromatogram at amounts varying between 0.7 and 6.9% AR at 24 hours. Identification of these fractions, however, was not pursued in this project.

In the dark control samples no degradation was found as indicated by MAB_{1a} concentrations of 99.3% and 97.8% at 0 and 24 hours, respectively.

Table 4.1.4.12-1. Photolysis of MAB_{1a} in aqueous buffer pH 7 during a 1-day exposure period in a Suntest apparatus (% AR, average of 2 replicates)

	Irradiation time (hours)						
	0	**1.5**	**3**	**5**	**6.5**	**10**	**24**
MAB_{1a}	99.3	96.6	86.7	86.1	87.9	69.1	44.6
Others[1]	1.7	6.6	15.1	13.5	14.4	29.0	55.0
Total	101.0	103.2	101.8	99.6	102.3	98.1	99.6

[1] comprise up to 18 fractions in HPLC chromatogram varying between 0.7 and 6.9% AR

The experimental half-life (DT_{50}) was calculated as 21 hours (= 0.89 days).

The quantum yield of MAB_{1a} was calculated as $\Phi = 1.44 \times 10^{-2}$ molecules degraded per photon. Based on the quantum yield, the environmental half-life of emamectin benzoate due to direct photolysis at the surface of pure water was calculated at 30° N, 40° N and 50° N latitude and in natural water (River Rhine, latitude 40° N) down to 30 cm depth using the program GCSOLAR[1].

Table 4.1.4.12-2: Theoretical half-life (days) of emamectin benzoate close to the surface of pure water and in River Rhine water down to 30cm depth calculated using GCSOLAR

	Spring	**Summer**	**Fall**	**Winter**
Pure water[1]				
Latitude 30° N	1.49	1.32	2.13	2.95
Latitude 40° N	1.63	1.35	2.81	4.69
Latitude 50° N	1.88	1.42	4.27	9.48
River Rhine[2]				
Depth 0 cm	1.63	1.35	2.81	4.69
Depth 10 cm	1.78	1.47	3.06	5.10
Depth 20 cm	1.93	1.60	3.33	5.53
Depth 30 cm	2.08	1.73	3.60	5.97

[1] close to the surface, longitude 10°, terrestrial type of atmosphere, typical ephemeride and ozone values;
[2] latitude 40° N

Conclusions

The half-life of MAB_{1a} under "Suntest" conditions was calculated as 21 hours. Quantum yield for the photochemical reaction was determined at 1.44×10^{-2} molecules degraded per photon. Based on the quantum yield, the half-lives of MAB_{1a} under aqueous conditions at latitudes between 30° N and 50° N were calculated to range from 1.32 to 9.48 days depending on the latitude and season. Accordingly, MAB_{1a} is considered to be rapidly photo chemically degraded in aqueous systems under natural sunlight.

Remarks

No identification of metabolites as these were < 10% or AR. Recalculation of the half-life under study conditions revealed an identical value.

4.1.4.12 Study 13 - Photochemical degradation in soil

Study reference: Anderson, W. (2003), STUDY IIA, 7.1.3/001

Detailed study summary and results

Reference/notifier	:	Anderson, W. (2003)	GLP statement	:	yes
Type of study	:	soil photolysis	Guideline	:	US-EPA 540/9-82-021, Section 161-3 95/36/EC
Year of execution	:	2002-2003	Acceptability	:	acceptable

[1] US EPA, GCSOLAR User's manual, Version 1.20, July 1999

Test substance	:	[23-^{14}C] –emamectin benzoate B_{1a}, batch CL-LIII-77, radiochemical purity 98.6 % [23-^{14}C] –emamectin benzoate B_{1b}, batch CL-LIII-80, radiochemical purity 98.7 %

Substance	Soil type	T [° C]	OM [%]	pH	Light source	Wavelength [nm]	Duration [d]	Transformation at end [%]	$DT_{50,photo}$ [d]
emamectin benzoate B_{1a}	sandy loam	25	0.5	8.3	Xenon	300-700	29	85.4	12.2
emamectin benzoate B_{1b}	sandy loam	25	0.5	8.3	Xenon	300-700	29	73.8	20.3

Description

Soil. Sandy loam (Maricopa, Arizona, USA), was 2 mm sieved and moisture content adjusted to 75 % FC. Physical chemical properties of the soil are reported in table 4.1.4.13-1.

Table 4.1.4.13-1. physicochemical characteristics of the soil used in the study

Syngenta ID	Arizona Sandy Loam
pH (1:1 soil:water ratio)	8.3
(saturated paste)	8.1
CEC (meq./100 g) [Ψ]	17.7
Organic Matter (%)	0.5
Olsen Phosphorous (ppm)	8
Total Nitrogen (%)	0.041
Soluble salts (mmhos/cm)	0.73
Moisture (%) at 1/3 Bar	16.0
Max. Water Holding Capacity	28.4
Sand (%)[λ]	68
Silt (%)[λ]	20
Clay (%)[λ]	12
Soil Classification (Hydrometer Method)	Sandy Loam
Bulk Density (g/cc)	1.46
Source (City, State, Country)	Maricopa Arizona

[Ψ] Cation exchange capacity- in milliequivalents/100 g of soil
[λ] The percent sand, silt and clay were determined using the hydrometer method.

Microbial biomass determined according to Anderson and Domsch (1978), was 99.9 µg C/g soil at the start of the study. No pesticides were used at the field site for the previous five years.

Method.

Soil samples (ca. 7 g dry weight) were put into 5 mL sterile vials with teflon lined caps and the test substance was added as a solution in ethanol, fortification levels 0.141 µg emamectin benzoate B_{1a}/g soil and 0.151 µg emamectin benzoate B_{1b}/g soil in separate samples. Samples selected for irradiation were placed on their sides in a water bath to allow for maximum exposure to the artificial light source. Irradiation was performed for 30 days with a xenon arc lamp emitting light at initial average daily intensity of ca. 4.41 x 10^{-3} W/cm^2 and a wavelength spectrum of 300 to 700 nm (wavelength ≤ 290 nm were filtered). Non-irradiated samples were wrapped in aluminium foil and placed in a constant temperature room. The temperature was maintained at 25 ±1°C for both irradiated and radiated samples. During the test period, the samples were continuously

purged (16 mL/min) for volatiles with sterile moist air via a silicone septum. Samples were taken on days 0, 2, 4, 7, 14, 21 and 30.

Analysis. The moist soil was transferred to a separate flask and extracted with acetonitrile:water (80:20, v/v) twice. Aliquots of the combined extracts (Extract 1) were radioassayed and subjected to 2D-TLC and/or HPLC for characterisation using reference standards.

If the percentage of the total dose was ≤ 93% after this extraction, the soil was extracted once more using acetonitrile:0.5M HCl (pH 6) (3:1, v/v) under the same conditions as described before (Extract 2). If the percentage of the total dose was ≤ 90% after the 2nd extraction, the soil was extracted once more using acetonitrile:0.5M HCl (pH 6) (3:1, v/v) heated to 60°C for 2 hours (Extract 3). After centrifugation, the soil was either air dried for combustion or subjected to organic matter fractionation. Only derivatives of MAB_{1a} were available as reference standards. Reference standards used were: emamectin benzoate B_{1b}, emamectin benzoate B_{1a}, MFB_{1a}, FAB_{1a}, MSB_{1a}, 8a -oxo MAB_{1a}, and AB_{1a}. If a degradate of emamectin benzoate B_{1b} co-chromatographed with one of the emamectin benzoate B_{1a} reference standards it was assumed that this is the emamectin benzoate B_{1b} analogue of that respective reference standard.

All samples were stored after harvest in the freezer at below -5°C for up to 4 weeks.

Calculations. The half-lives for both test compounds under either condition were calculated using first order kinetics and Excel and Origin software.

Results

Microbial biomass of the soil at the end of the study was 104.0 µg C/g soil. The total average radiochemical balance ranged from 98.429% to 109.00% of the total dose for MAB1a, and from 96.52% to 106.92% of the total dose for MAB_{1b}.

2D-TLC analysis of deep-frozen samples indicated that there was minimum degradation during freezer storage.

The limit of quantification ranged from 0.00032 to 0.00063 µg and from 0.00026 to 0.00063 µg for all assay types for samples dosed with ^{14}C-MAB_{1a} and ^{14}C-MAB_{1b}, respectively.

Distribution of radioactivity in irradiated and dark samples is given in Table 4.1.4.13-2.

Table 4.1.4.13-2. Recovery and distribution of radioactivity after application of MAB_{1a} to soil and irradiation / non-irradiation (% AR, average of 2 replicates)

Time	Volatiles	Extract 1	MAB_{1a} &	AB_{1a} &	MFB_{1a} &	FAB_{1a} &	Zone 12&	Total non identified fraction&	Extract 2	Extract 3	Non-extractable	Total
Irradiated												
0 d	n.p.	98.05	95.27	<LOQ	n.d.	n.d.	n.d.	n.d.	n.p.	<LOQ	3.44	101.49
2 d	1.96	100.95	75.61	n.d.	1.17	1.52	3.26	3.12	n.p.	<LOQ	6.10	109.00
4 d	3.49	82.10	49.29	**7.95**& &	**2.11**	2.17	4.22	11.44	5.50	1.99&&	6.33	98.42
7 d	3.41	78.67	41.26	n.d.	1.62	1.86	**7.93**	13.87	6.73	2.66	7.39	98.85
14 d	7.28	70.27	29.19	n.d.	1.17	1.97	5.38	15.13	6.63	2.70	12.11	98.97
21 d	9.27	71.95	26.18	n.d.	1.88	**3.02**	5.12	**24.71**	4.22	2.69&&	21.12	107.9
30 d	**9.48**	55.28	14.62	2.39	1.76	2.16	2.74	16.12	5.03	4.01	**26.02**	99.81
Non-irradiated												
0 d	n.p.	104.63	97.44	n.d.	n.d.	n.d.	n.d.	n.d.	n.p.	<LOQ	3.34	107.97
2 d	<LOQ	95.20	82.54	n.d.	<LOQ	<LOQ	3.13	n.d.	4.13&&	<LOQ	3.87&&	99.200
4 d	5.75&&	97.79	90.76	n.d.	1.72	<LOQ	2.74	n.d.	n.p.	<LOQ	4.55	105.21
7 d	3.06	98.34	80.18	n.d.	**3.89**	1.59	7.43	7.45	6.43&&	<LOQ	4.46	109.0

Time	Volatiles	Extract 1	MAB_{1a} [&]	AB_{1a} [&]	MFB_{1a} [&]	FAB_{1a} [&]	Zone 12[&]	Total non identified fraction[&]	Extract 2	Extract 3	Non-extractable	Total
												8
14 d	**9.19**	84.60	64.26	n.d.	2.46	1.13	4.19	n.d.	5.08	<LOQ	2.88	101.74
21 d	4.00	84.82	55.94	1.21[&&]	2.41	1.87	7.92	2.56	5.36	n.p.	5.88	100.05
30 d	8.53	84.78	48.34	**1.85**	1.86	**3.42**	**8.32**	**12.79**	6.60[&&]	n.p.	**11.56**	108.17

[&] Based on analysis of Extract 1; [&&] detected in a single replicate;
n.p. = not performed; n.d. = not detected; LOQ = limit of quantification

Table 4.1.4.13-3. Recovery and distribution of radioactivity after application of MAB_{1b} (NOA 422390) to soil and irradiation / non-irradiation (% AR, average of 2 replicates)

Time	Volatiles	Extract 1	MAB_{1b} [&]	MAB_{1b} analogue of AB_{1a} [&]	MAB_{1b} analogue of MFB_{1a} [&]	MAB_{1b} analogue of FAB_{1a} [&]	Zone 12[&]	Total non identified fraction[&]	Extract 2	Extract 3	Non-extractable	Total
Irradiated												
0 d	n.p.	103.28	93.03	<LOQ	n.d.	n.d.	5.14[&&]	2.13	n.p.	<LOQ	3.64	106.92
2 d	1.38[&&]	92.38	68.44	n.d.	n.d.	n.d.	n.d.	1.09	5.35	<LOQ	3.33	101.74
4 d	5.95[&&]	78.88[&&]	47.92	n.d.	1.94	2.13	5.05	10.92	5.29[&&]	1.77[&&]	6.07[&&]	97.96[&&]
7 d	5.74	83.06	41.13	n.d.	1.98	**2.44**	5.56	22.48	6.24	2.95[&&]	6.49	103.00
14 d	13.38	72.74	30.52	**2.91**[&&]	**2.64**	2.09	5.04	**24.54**	6.43	2.59	10.23	105.37
21 d	7.93	72.83	34.28	n.d.	2.31	2.36	**7.95**	11.84	6.74	2.53[&&]	7.89	98.88
30 d	**14.43**	60.03	26.16	n.d.	1.50	1.99	7.33	10.35	4.80	2.65	**13.22**	100.27
Non-irradiated												
0 d	n.p.	100.15	88.66	n.d.	n.d.	n.d.	4.92	n.d.	n.p.	<LOQ	3.51	103.66
2 d	1.08	100.61	95.22	n.d.	n.d.	n.d.	n.d.	1.79	n.p.	<LOQ	4.39	106.07
4 d	2.93[&&]	97.96	83.93	n.d.	<LOQ	<LOQ	4.21	1.07	n.p.	<LOQ	5.06	104.48
7 d	1.82	96.91	78.85	<LOQ	**2.52**[&&]	1.44[&&]	6.10	5.19	5.52[&&]	n.p.	**5.48**	106.96
14 d	1.49	89.20	65.36	n.d.	1.47	1.44[&&]	5.20	4.24	5.22	<LOQ	3.13	99.03
21 d	3.03	96.27	68.61	1.14[&&]	2.42	1.32	7.78	1.80	5.26	n.p.	3.73	105.29
30 d	**3.65**	78.85	48.36	**1.71**[&&]	1.95[&&]	**2.46**[&&]	**8.20**	7.47	6.70	2.38	4.95	96.52

[&] Based on analysis of Extract 1; [&&] detected in a single replicate;
n.p. = not performed; n.d. = not detected; LOQ = limit of quantification

Zone 12 reached maximum concentrations of >5% of applied RA at two consecutive timepoints. In study 7.1.1/003 and 7.2.1.3.2/002 zone 12 has been detected and its structure has been proposed as the carboxylic acid of the cleaved macrocycle.

The total non-identified fraction at day 21 consists of several compounds with a maximum percentage of 4.5%. For MAB_{1a} the results are given in table Table 4.1.4.13-4 below. For MAB_{1b} a similar picture for unidentified compounds is shown, with none of the individual compounds exceeding 5%.

Table 4.1.4.13-4. non identified fraction of radioactivity after application of MAB_{1a} to soil and irradiation / non-irradiation (% AR, average of 2 replicates)

	Day 21	Unidentified component	Identified component
Component	**Avg.**	**Avg.**	
A, NOA-426007	26.18	-	MAB_{1a}
	ND	-	AB_{1a}
D, NOA-438307	<MQA	-	
E, NOA-438309	1.88	-	MFB_{1a}
	3.02	-	FAB_{1a}
F, NOA-415692	4.50	4.50	-
	2.61	2.61	-
G, NOA-415693	5.12	-	Zone 12
5	2.15	2.15	-
6	1.57	1.57	-
12	2.79	2.79	-
14	2.44	2.44	-
23	1.51	1.51	-
28	1.57	1.57	-
29	3.86	3.86	-
30	1.71	1.71	-
45			
48			
Origin			
Total unidentified radioactivity (as percent total dose)		24.71	

Although the irradiated sample vials were capped tightly and sealed with both glue and parafilm before being placed in the waterbath, some water did manage to leak into some of the vials. The effect of the added moisture appeared to be an increase in volatiles and an increase in level of non-extractable residues. Despite this variation, the overall degradation rates and degradation products were similar throughout the study for both MAB_{1a} and MAB_{1b}.

Reported DT_{50} values are summarised in the following table.

Table 4.1.4.13-4. Half-life of MAB_{1a} and MAB_{1b} on soil

	MAB_{1a} (NOA 426007)		MAB_{1b} (NOA 422390)	
	Half-life (days)	Correlation r^2	Half-life (days)	Correlation r^2
Irradiated	12.41	0.960	19.62	0.863
Non-irradiated	29.95	0.980	33.75	0.954
Corrected	21.13	n.a.	46.83	n.a.

	MAB_{1a} (NOA 426007)		MAB_{1b} (NOA 422390)	
net-photolysis				

Recalcultation of DT_{50} values later in time using simple first order kinetics in ModelManager resulted in different values as reported in table 4.1.4.13-5.

Table 4.1.4.13-5. Half-life of MAB_{1a} and MAB_{1b} on soil

	NOA426007		NOA422390	
	DT50 (DAR)	DT50 (Syngenta SFO)	DT50 (DAR)	DT50 (Syngenta SFO)
Irradiated	8.57	8.57 (0.809)	12.63	12.93 (0.0536)
Non-irradiated	28.97	28.97 (0.0239)	35.65	35.64 (0.0194)
Corrected net photolytic DT50	49.06	12.2 (0.0570)	50.23	20.3 (0.0342)

k values in (), where k = the rate constant, and DT50 = ln(2)/k

rate of photolysis alone = k (irradiated) - k (dark control)

Remarks

Reference standards used did not include soil metabolites 8a-OH MAB_{1a}, aglycone and N-nitroso MAB_{1a}. The total fraction non-identified radioactivity from extract 1 reached maximum rate of 24.7% under irradiated conditions,non of the indivual compounds reached 5% however. DT_{50}-values were recalculated by non-linear fit of first order kinetics.

4.1.4.13 Study 14 - Photochemical degradation in soil

Study reference: Chukwudebe (1994b), STUDY IIA, 7.1.3/002

Detailed study summary and results

Reference/notifier	:	Chukwudebe (1994b)	GLP statement	:	yes
Type of study	:	soil photolysis	Guideline	:	EPA Subdivision N, Section 161-3, 1982
Year of execution	:	1992-1993	Acceptability	:	acceptable
Test substance	:	^{14}C- emamectin benzoate B_{1a} , radiochemical purity 98.6 %			

Substance	Soil type	T	OM	pH	Light source	Wavelength	Duration	Transformation at end	$DT_{50,photo}$
		[° C]	[%]			[nm]	[d]	[%]	[d]
^{14}C- emamectin benzoate B_{1a}	sandy loam	23.2-27.8	2.6	7.8	artificial	>290 nm	30	96	3.9

Description

Soil. Sandy loam (Buckeystown, MD, USA), 2 mm sieved, brought to 75% FMC (pF 2.5) prior to start of the experiment.

Table 4.1.4.14-1. physicochemical characteristics of the soil used in the study

Soil Source	Buckeystown, Frederick Co., MD
Order	Ultisol
Series	Sequatchie
Horizon	A (0 - 6")
Texture (USDA)	Sandy Loam
% Sand	71
% Silt	12
% Clay	17
Organic Matter (%)	2.6
pH	7.8
% Field Moisture Capacity (FMC) at ⅓ Bar	23.1
Cation Exchange Capacity (meq/100g)	10.6
Bulk Density (g/mL)	1.16

Microbial viability: > 5.0 x 10^8 CFU/g soil at the start of the study. Soil was obtained from a field that was known to be under active farming with crops of corn or soybeans produced over the past several years.

Method.

Soil samples (thickness of approximately 2 mm) were put into glass dishes (inner diameter 45 mm) and the test substance was added as a solution in ethanol, fortification level 5.81 µg emamectin benzoate B_{1a}/g soil. Dishes were tightly sealed and had an inlet and outlet for collecting volatiles. Volatile traps: ethylene glycol, 1 N H_2SO_4 and KOH solutions. Samples selected for irradiation were placed in a Suntest apparatus equiped with a xenon lamp emitting light at initial average daily intensity of 400-765 W/m^2 (wavelength ≤ 290 nm were filtered). Non-irradiated samples were wrapped in aluminium foil and placed in a constant temperature room. The temperature was maintained at 25 ±1°C for both irradiated and radiated samples. Incubation was performed with a 12:12 hours light:dark cycle. Duplicate samples of irradiated soil and single samples of non-irradiated soil were taken on days 0, 2, 4, 7, 14, 21 and 30.

Analysis. The moist soil was extracted with methanol containing 100mM ammonium acetate. The extract was decanted and the soil was extracted twice with methanol:water (4:1, v/v) containing 100mM ammonium acetate. Aliquots of the combined extracts (Extract 1) were radioassayed and subjected to HPLC for quantification of ^{14}C-components including parent compound. All soils from day 7 to day 30 were subjected to two subsequent extractions using methanol:water (1:5, v/v) containing 100mM ammonium acetate. The extracts were combined (Extract 2) and the radioactivity in duplicate aliquots was quantified by LSC. Since the radioactivity was < 5% AR, residual soils were extracted three times with ethyl acetate:ammonium hydroxide (10:1, v/v). The extracts were combined (Extract 3) and aliquots were subjected to LSC. HPLC analysis of Extract 3 was done together with the analysis of Extract 1 using unlabelled MAB_{1a} as the only reference substance. Following solvent extractions, all soils were combusted and radioactivity quantified by LSC.

No soil samples were stored prior to extraction. Stability of MAB_{1a} residues in Extract 1 under storage conditions at –20°C was demonstrated by spiking selected extracts. MAB_{1a} was demonstrated to be not stable in Extract 3. However, this was not considered to be a problem for half-life calculation since MAB_{1a} was previously extracted from the soils by Extractions 1 and 2.

Additional soil samples exposed under similar conditions as described before were used for in-depth characterisation of the residues resulting from this soil photodegradation study. At each sampling interval, four replicate soil samples were composited, mixed with 10 mL methanol containing 100mM ammonium acetate and stored deep-frozen until extraction and analysis. The extraction procedure was equivalent to that described before except that the first extraction step (MeOH + 100mM NH4Ac) was repeated 6 times followed by a single extraction with MeOH:H_2O (4:1, v/v). The supernatants of these seven extractions were combined. The soil was then extracted 15 times with ethyl acetate:ammonium hydroxide (10:1, v/v) and the extracts were combined. Prior to the determination of the residues profiles in the proportionately pooled extract, the extract was evaporate to dryness using a nitrogen stream and then reconstituted in MeOH:H_2O

(1:1, v/v). Each extraction step was accompanied by quantification of radioactivity using LSC. The radioactivity in the post-extracted soil was quantified by LSC following combustion.

The pooled extract was subjected to broad fractionation of the extracted residues into polar and non-polar fractions using solid phase extraction (SPE). Total soil extracts as well as the polar and non-polar fractions generated by SPE were submitted to HPLC analysis for characterisation of the soil photodegradation residues.

Calculations. The half-life for MAB_{1a} under either condition was calculated based on logarithmic average residue values using linear regression. The slope of the best-fit line is equivalent to 'k' in the formula: half-life = ln2 / k.

Results

The measured light intensity during the study on average 0.1049 W/cm^2.

The total average radiochemical balance ranged from 92.9% to 108.6% AR for the irradiated samples and from 95.6% to 110.7% AR for the dark control samples. Microbial viability at end of the experiment: > 6.5 x 10^8 CFU/g soil, which is indicative of a viable population. The limit of detection ranged from 0.0032 to 0.0065 ppm for all assay types.

Distribution of radioactivity in irradiated and dark samples is given in Table 4.1.4.14-2.

Table 4.1.4.14-2. Recovery and distribution of radioactivity after application of MAB_{1a} to soil and irradiation / non-irradiation (% of applied radioactivity)

Time	Total volatiles	CO_2	Extract 1	Extract 2	Extract 3	MAB_{1a} [&]	Fraction 1[&]	Fraction 2[&]	Fraction 3[&]	Fraction 4[&]	Non-extractable	Total
Irradiated (average of 2 replicates)												
0 d	n.p.	n.p.	99.1	n.p.	n.p.	99.1	0.0	0.0	0.0	0.0	0.0	99.1
2 d	1.8	1.8	99.1	n.p.	n.p.	70.7	7.1	5.6	3.7	8.1	7.3	104.2
7 d	3.7	3.0	80.7	2.3	7.6	39.9	18.1	7.0	5.5	10.4	14.5	108.6
14 d	8.9	8.9	58.3	3.1	5.2	13.7	25.5	7.0	4.7	7.4	22.4	97.8
21 d	10.2	10.2	46.3	4.1	4.4	6.9	22.0	7.5	5.6	4.3	30.5	95.9
30 d	8.5	6.8	39.2	3.31	9.9	2.5	24.5	9.2	6.8	6.0	32.1	92.9
Non-irradiated (1 replicate)												
2 d	1.4	1.4	100.8	n.p.	n.p.	92.8	0.0	1.1	0.6	6.2	3.4	105.6
7 d	3.8	2.6	85.6	1.9	8.6	55.1	13.6	5.8	5.6	14.0	10.8	110.7
14 d	13.0	13.0	62.6	2.6	5.5	24.4	19.9	2.9	6.0	9.4	21.0	104.7
21 d	9.5	9.5	57.8	2.9	5.4	13.9	20.5	6.9	6.4	10.1	21.5	97.1
30 d	9.3	9.3	47.0	2.5	10.9	8.5	22.7	8.6	7.9	10.1	24.5	95.6

[&] Based on analysis of Extract 1; [&&] detected in a single replicate;
n.p. = not performed; n.d. = not detected; LOQ = limit of quantification

RP-HPLC analysis of extracted residues showed that the highest radioactivity corresponding to non-polar residues was present in the 21-day sample. Therefore, additional non-polar fractions were generated by SPE from this sample for further characterisation by NP-HPLC.

Reference standards used were: emamectin benzoate, MSB_{1a}, FAB_{1a}, MFB_{1a}, 8a -oxo MAB_{1a}, 8a-OH MAB_{1a}, AB_{1a} and 8,9-Z-MAB_{1a}. The findings of RP- and NP-HPLC analyses of the non-polar fraction are summarised in the following table.

Table4.1.4.14-3. Quantitative distribution of non-polar fraction components in MAB_{1a} treated soil following 21-day photoirradation (% of applied radioactivity)

MAB_{1a}	8,9Z MAB_{1a}	AB_{1a}	8a-OH-MAB_{1a}	8a-oxo-MAB_{1a}	MFB_{1a}	FAB_{1a}	MSB_{1a}
28.06	0.49	1.97	3.18	1.11	0.73	0.26	0.19

Reported DT_{50} values in the original report are summarised in the following table:

Table 4.1.4.14-4. Half-life of MAB_{1a} on soil

	Half-life (days)	Rate 'k'	Correlation r^2
Irradiated	5.3	-0.1321	0.963
Non-irradiated	8.0	-0.0868	0.991
Corrected net-photolysis	15.3	-0.0453	n.a.

On a later timepoint additional dat on the study were submitted.

The study was set up with light/dark regime on a 12 hours cycle. The light received by the samples is converted to natural day equivalents using the following equation:

Days= (h x r_x)/(0.75 x 12)

Where:

H=hours of Suntest irradiation

r_x = ratio of Suntest intensity to natural sunlight intensity

0.75= correction factor for time of day dependence

12= conversion of hours to days (assuming 1 natural day has 12 hours sunlight)

The light intensity values in Table V of the original report enable the intensity ratio (r_x) to be calculated.

Table 4.1.4.14-5

Latitude	Natural Intensity (300 - 400 nm): W m^{-2}	r_x
50°N	57.34 (*)	0.25
40°N	59.54 (*)	0.24
30°N	60.14 (*)	0.24
Measurement at Agrisearch	37.24	0.38
Xenon lamp intensity	**14.26**	

(*) Default assumption calculated using the T. Mill *et al* data (in Lλ) as published in the OECD guidance document 7 (published February 1997)

The days equivalent of natural sunlight at various latitudes can then be calculated:

Table 4.1.4.14-6 equivalent dayss of natural sunlight

Number of Experimental Days (12 hrs light/dark cycle)	Actual Number of Hours of Suntest Irradiation	Days Equivalents of summer irradiation at 50^0N	Days Equivalents of summer irradiation at 40^0N	Days Equivalents of summer irradiation at 30^0N	Days Equivalents of summer irradiation at Agrisearch
0	0	0.00	0.00	0.00	0.00
2	24	0.66	0.64	0.63	1.02
7	84	2.32	2.23	2.21	3.57
14	168	4.64	4.47	4.42	7.15
21	252	6.96	6.70	6.64	10.72
30	360	9.94	9.58	9.48	15.31

For the estimation of the photolytic half-life of the test item, the light intensity received by the samples under the Suntest can firstly be converted to days equivalent of summer sunlight for the various latitudes prior to modelling. However, to minimise incremental errors on calculation, the percentage of applied test item were plotted against hours of continuous irradiation under the Suntest, fitted to a simple first order (SFO) kinetic model, to estimate the T½. The resultant DT_{50} values were corrected to days of natural summer sunlight at 30, 40 and 50°N using correction factors.

The DT_{50} for both the irradiated samples and the dark controls were re-calculated using Simple First Order kinetics:

Table 4.1.4.14-7 recalculated half lifes

Irradiated DT_{50} (SFO)	2.6 days
k (Irradiated)	0.2696
Dark control DT_{50} (SFO)	7.4 days
k (Dark control)	0.0931
DT_{50} (due to photolysis alone)*	3.9 days
k (due to photolysis alone)	0.1765

SFO - Single first order kinetics
Irradiated DT_{50} calculated based on the hours of "continuous" irradiation (converted to 24 hrs day). i.e. 2 experimental days (of 12 hrs dark/light cycle) = 24 hrs continuous irradiation = 1 days artificial Xenon lamp
k - first order rate constant, $DT_{50} = \ln(2)/k$
* - rate of photolysis alone = k (irradiated) - k (dark control)

The photolytic DT_{50} can be converted to equivalent days of natural sunlight days:

Table 4.1.4.14-8 Converted photolysis half-life

Latitude	T 1/2 (Days Continuous)	Correction Factor	Corrected T 1/2
30^oN	3.9	0.63	2.5
40^oN	3.9	0.64	2.5
50^oN	3.9	0.66	2.6
Agrisearch	3.9	1.02	4.0

The rate of degradation due to photolysis alone (DT_{50} 2.5 - 4 days) was faster than the dark control (7.4 days), hence photolysis may represent a potentially significant pathway for degradation of emamectin in the environment.

Remarks

It is reported that soil was obtained from a field that was known to be under active farming over the past several years. No information on pesticide use is reported. The Suntest is set to give the same amount of intensity so the ratio of the Suntest to natural sunlight is ~1 (for ease of experimental timing). However, it should be noted that the actual intensity for the Suntest was less than the natural intensity (Figures 7). Hence "real" photolytic DT_{50} could be faster than reported. But there is not sufficient data reported to carry out conversion.

4.1.4.14 Study 15 - Field dissipation

Study reference: Evans, P.G. (2006), STUDY IIA, 7.3.1/001

Detailed study summary and results

Reference/notifier	:	Evans, P.G. (2006)	GLP statement	:	yes
Type of study	:	field dissipation, final report	Guideline	:	not specified
Year of execution	:	2005	Acceptability	:	acceptable
Test substance	:	A10324A, a 50 g/kg SG formulation, containing emamectin benzoate, 4.89%			

Substance	Location	Soil type	Land use	Dose [g as/ha]	Date of application	OM [%]	pH[&]	Duration [d]	$DT_{50,field}$ emamectin benzoate B_{1a} [d]
A10324A	Tiercé	sandy loam	grown sparsely with grass	22.5	13-06-2005	2.2	5.6	177	2.3

[&] in 0.01M $CaCl_2$

Description

Location. Tiercé (France), sandy loam, CEC 89 mmol/kg. Field grown sparsely with grass. For a period of up to 3 years prior to the trial, different arable crops were grown on the test site and maintained following common local agronomical practice, however, no emamectin benzoate was applied. During the test period, no additional chemicals, fertilisers and nutrients were used

Application and plot maintenance. A single unreplicated plot was used: 30 m long and 6.6 m wide to give a treatment plot of 199.8 m^2. The plot was divided into four subplots of 7 m length leaving 1-m boundary strips at the top and low end of the plot, which was sampled for soil characterisation using a zero contamination corer. Grass was sown seven days before application, resulting in sparse grass cover. The plot was maintained by keeping the grass cover below a height of 20 cm through regular mowing throughout the study period. Test substance was applied by broadcast spray on 13 June 2005 in a single treatment of 0.45 kg product/ha in water at 500 L/ha, equivalent to 22.5 g as./ha. Weather conditions at time of application: dry weather, 22.4 °C, wind speed 0.8-1.8 m/s during application. The application rate was verified.

Sampling. Samples were taken from the control plot four days prior to application and on the day of application. Samples from the treated plot immediately after application and on days 1, 2, 3, 7, 15, 30, 59, 120 and 177 days after application. Sampling with a 5 cm diameter corer, 30 cm depth, 20 cores per sampling date. Cores were deep-frozen and cut into 10 cm layers, corresponding depths were pooled and homogenised with dry ice. Samples were stored deep-frozen for up to 8 months prior to analysis.

Analysis. Soil samples were analysed according to method RAM 475/01. Soil is extracted with acetonitrile:100 mM ammonium acetate acid solution (80:20, v/v) using mechanical agitation. After centrifugation, an aliquot of the soil extract is diluted with acetonitrile/water solution (20:80, v/v) prior to analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS). The limit of quantification of the method is 0.0005 mg/kg.

Reference substances: emamectin benzoate B_{1a}, emamectin benzoate B_{1b}, 8,9-Z-MAB_{1a}, and 8a-OH MAB_{1a}. Concurrent (mean) recovery was 81 - 95 % at levels of 0.0005, 0.001, 0.01 and 0.10 mg/kg.

Calculations. Dissipation rate constants were calculated using both simple first order (SFO) and first order multi compartment (FOMC) models.

Results

Weather data. Weather data are summarised in the following table.

Table 4.1.4.15-1. Weather conditions during the study

	T [°C] min/max/avg	Rainfall [mm]	Irrigation [mm]
June 2005[&]	7.3 / 35.4 / 21.4	32.4	0
July 2005	9.1 / 36.1 / 22.6	24.6	0
Aug-2005	7.5 / 34.6 / 21.1	22.8	0
Sep-2005	4.8 / 32.2 / 18.5	17.4	122
Oct-2005	5.6 / 26.8 / 16.2	112.2	39
Nov-2005	-3.1 / 18.9 / 7.9	47.2	0
Dec-2005[&]	1.3 / 12.4 / 6.9	26.6	0

[&] weather data available only for part of the month

Residues.

No residues of MAB_{1a} and the other analytes were found at the test site prior to the application treatment. Deposition tray soil analysis on basis of MAB_{1a} residues showed a mean recovery of 49% of the expected residue after treatment. Taking into account the high sorption properties of emamectin benzoate to soil, this values indicates that the correct rate of the formulated product was applied.

Table 4.1.4.15-2: Theoretical and measured initial concentration of Emamectin benzoate 05 SG in the deposition tray soil samples (n = 5)

Test site	Application rate (g ai/ha)	Theoretical rate of MAB_{1a}[&] (g/ha)	Initial measured residue – mean (range) (g/ha)	Percentage related to theoretical value – mean (range)
Tiercé (F)	22.5	20.25	10.5 (4.5 – 15)	52 (22 - 72)

Since for none of the analytes residues > LOQ were found in the 10-20 cm layer, samples of the 20-30 cm layer were not analysed at all.

The residue values of MAB_{1a} and MAB_{1b} in the top 0-10 cm layer are summarised in Table 4.1.4.15-3.

Table. 4.1.4.15-3. Residue levels (mg ai/kg soil) of MAB_{1a} and MAB_{1b} in the 0-10 cm soil layer (wet weight)

Days	Tiercé (F)	
	MAB_{1a}	MAB_{1b}
-4 to 0 (PA)	<LOQ	<LOQ
0	0.0083	0.0006
1	0.0092	0.0006
2	0.0034	<LOQ

3	0.0033	<LOQ
4	n.s.	n.s.
7	0.0016	<LOQ
14	n.s.	n.s.
15	<LOQ	<LOQ
21	n.s.	n.s.
28	n.s.	n.s.
30	<LOQ	<LOQ
58	n.s.	n.s.
59	<LOQ	<LOQ
60	n.s.	n.s.
112	n.s.	n.s.
120	<LOQ	<LOQ
171	n.s.	n.s.
174	n.s.	n.s.
177	<LOQ	<LOQ
181	n.s.	n.s.
350	n.s.	n.s.
358	n.s.	n.s.

n.s. = not sampled

No residues of 8,9-Z-MAB_{1a}, and 8a-OH $MAB_{1a,}$ above the limit of quantification (0.0005 mg/kg) were determined in any soil horizon at any analysis interval during the study.

DT_{50} values for emamectin benzoate B_{1a} reported by the author are summarised in the following table. FOMC fitting did not result in a better fit, the SFO model was appropriate to fit the data.

Table. 4.1.4.15-4. emamectin benzoate B_{1a} DT_{50} values in sandy loam soil.

Test site	Simple first order (SFO) model			
	DT_{50} (d)	DT_{90} (d)	r^2	χ^2
Tiercé (F)	2.56	8.49	0.730	23

Remarks

The dissipation half-life can be accepted.

4.1.4.15 Study 16 - Field dissipation

Study reference: Evans, P.G. (2006), STUDY IIA, 7.3.1/002

Detailed study summary and results

Reference/notifier	:	Evans, P.G. (2006)	GLP statement	:	yes
Type of study	:	field dissipation, final report	Guideline	:	not specified
Year of execution	:	2005-2006	Acceptability	:	acceptable
Test substance	:	A10324A, a 50 g/kg SG formulation, containing emamectin benzoate, 4.89%			

Substance	Location	Soil type	Land use	Dose	Date of application	OM	pH[&]	Duration	$DT_{50,field}$ emamectin benzoate B_{1a}
				[g as/ha]		[%]		[d]	[d]
A10324A	Marsillargues	silty clay loam	grass covered	22.5	13-06-2005	1.9	7.4	177	0.8

[&] in 0.01M $CaCl_2$

Description

Location. Marsillargues (France), silty clay loam, CEC 156 mmol/kg. Field grown sparsely with grass. For a period of up to 3 years prior to the trial, different arable crops were grown on the test site. Crops grown were peas (2002), winter hard wheat (2003) and peas (2004). Chemicals used: glyphosate and bentazone. No emamectin benzoate was applied.

Application and plot maintenance. A single unreplicated plot was used: 30 m long and 6.0 m wide to give a treatment plot of 180 m^2. Grass was sown five days before application, resulting in sparse grass cover. The plots was maintained by keeping the grass cover below a height of 20 cm through regular mowing throughout the study period. Test substance was applied by broadcast spray on 7 June 2005 in a single treatment of 0.45 kg product/ha in water at 500 L/ha, equivalent to 22.5 g as/ha. Weather conditions at time of application: dry weather, 18.1 °C, wind speed 0.5-0.8 m/s during application. The application rate was verified.

Sampling. Samples were taken from the control plot four days prior to application and on the day of application. Samples were taken from the treated plot immediately after application and on days 1, 2, 3, 7, 15, 30, 59, 120 and 174 days after application. Sampling with a 5 cm diameter corer, 30 cm depth, 20 cores per sampling date. Cores were deep frozen and cut into 10 cm layers, corresponding depths were pooled and homogenised with dry ice. Samples were stored deep frozen for up to 8 months prior to analysis.

Analysis. Soil samples were analysed according to method RAM 475/01. Soil is extracted with acetonitrile:100 mM ammonium acetate acid solution (80:20, v/v) using mechanical agitation. After centrifugation, an aliquot of the soil extract is diluted with acetonitrile/water solution (20:80, v/v) prior to analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS). The limit of quantification of the method is 0.0005 mg/kg.

Reference substances: emamectin benzoate B_{1a}, emamectin benzoate B_{1b}, 8,9-Z-MAB_{1a}, and 8a-OH MAB_{1a}. Concurrent (mean) recovery was 90 - 104 % at 0.0005, 0.001, 0.01, 0.02 and 0.10 mg/kg.

Calculations. Dissipation rate constants were calculated using both simple first order (SFO) and first order multi compartment (FOMC) models.

Results

Weather data. Weather data are summarised in the following table.

Table 4.1.4.16-1. Weather conditions during the study

	T [°C] min/max/avg	**Rainfall [mm]**	**Irrigation [mm]**
June 2005[&]	7.7 / 34.2 / 21.0	18	0
July 2005	11.3 / 35.6 / 23.5	3.6	36
aug-2005	13.5 / 35.5 / 24.5	10.6	0
sep-2005	7.8 / 31.6 / 19.7	280.5	0
oct-2005	7.1 / 24.8 / 16.0	100.9	0
nov-2005[&]	-5.2 / 22.1 / 8.5	73.2	0

[&] weather data available only for part of the month

Residues

No residues of MAB_{1a} and the other analytes were found at any test site prior to the application treatment. Deposition tray soil analysis on basis of MAB_{1a} residues showed a mean recovery of 61% of the expected residues after treatment (cf. Table IIA 7.3.1-6).

Table 4.1.4.16-2. Theoretical and measured initial concentration of Emamectin benzoate 05 SG in the deposition tray soil samples (n = 5)

Test site	Application rate (g ai/ha)	Theoretical rate of MAB_{1a} [&] (g/ha)	Initial measured residue – mean (range) (g/ha)	Percentage related to theoretical value – mean (range)
Marsillargues (F)	22.5	20.25	12.4 (8 – 16)	61 (40 – 78)

Since for none of the analytes residues >LOQ were found in the 10-20 cm layer, samples of the 20-30 cm layer were not analysed at all.

The residue values of MAB_{1a} and MAB_{1b} in the top 0-10 cm layer are summarised in Table. 4.1.4.16-3.

Table. 4.1.4.16-3. Residue levels (mg ai/kg soil) of MAB_{1a} and MAB_{1b} in the 0-10 cm soil layer (wet weight)

Days	Marsillargues (F)	
	MAB_{1a}	MAB_{1b}
-4 to 0 (PA)	<LOQ	<LOQ
0	0.0096	0.0006
1	0.0017	<LOQ
2	0.0049	<LOQ
3	0.0011	<LOQ
4	n.s.	n.s.
7	0.0006	<LOQ
14	0.0012	<LOQ
15	n.s.	n.s.
21	n.s.	n.s.
28	n.s.	n.s.
30	0.0012	<LOQ
58	n.s.	n.s.
59	0.0008	<LOQ
60	n.s.	n.s.
112	n.s.	n.s.
120	0.0006	<LOQ
171	n.s.	n.s.
174	0.0006	<LOQ
177	n.s.	n.s.
181	n.s.	n.s.
350	n.s.	n.s.
358	n.s.	n.s.

n.s. = not sampled

No residues of 8,9-Z-MAB_{1a}, and 8a-OH $MAB_{1a,}$ above the limit of quantification (0.0005 mg/kg) were determined in any soil horizon at any analysis interval during the study.

DT_{50} values for emamectin benzoate B_{1a} reported by the author are summarised in the following table.

Table. 4.1.4.16-4. emamectin benzoate B_{1a} DT_{50} values in silty clay loam soil.

Test site	Simple first order (SFO) model				First order multi compartment (FOMC) model			
	DT_{50} (d)	DT_{90} (d)	r^2	χ^2	DT_{50} (d)	DT_{90} (d)	r^2	χ^2
Marsillargues (F)	0.847	2.81	0.768	51	0.087 RMS: 1	32.3 RMS: 3.4	0.912 nc	n.r. 52

nc: not calculated
nr: not reported

Remarks

DT_{50} for emamectin benzoate B_{1a} is recalculated by RMS according to SFO kinetics using residue levels reported in Table 4.1.4.16-4. The FOMC calculation by the author could not be reproduced with ModelMaker. The result $DT_{50,field}$ 0.8 days for emamectin benzoate B_{1a} is acceptable.

4.1.4.16 Study 17 - Field dissipation

Study reference: Seville, A.G. (2006a), STUDY IIA, 7.3.1/003

Detailed study summary and results

Reference/notifier	:	Seville, A.G. (2006a)	GLP statement	:	yes
Type of study	:	field dissipation, final report	Guideline	:	not specified
Year of execution	:	2004-2005	Acceptability	:	acceptable
Test substance	:	A10324A, a 50 g/kg SG formulation, containing emamectin benzoate, 4.89%			

Substance	Location	Soil type	Land use	Dose [g as/ha]	Date of application	OM [%]	pH[&]	Duration [d]	$DT_{50,field}$ emamectin benzoate B_{1a} [d]
A10324A	Grisolles	clay loam	sparsely covered with grass	22.5	25.05.2004	1.5	7.6	358	0.3

[&] in 0.01M $CaCl_2$

Description

Location. Grisolles (France), clay loam, CEC 124 mmol/kg. Field grown sparsely with grass. For a period of up to 3 years prior to the trial, different arable crops were grown on the test site. No crops were grown in 2004 prior to the study.

Application and plot maintenance. A single unreplicated plot was used: 30 m long and 6.0 m wide to give a treatment plot of 180 m^2. Grass was sown six days before application, resulting in sparse grass cover. The plot was maintained by keeping the grass cover below a height of 20 cm through regular mowing throughout the study period. Test substance was applied by broadcast spray on 25 may 2004 in a single treatment of 0.45 kg product/ha in water at 500 L/ha, equivalent to 22.5 g as./ha. Weather conditions at time of application: dry weather, 24 °C, wind speed 0.0-0.8 m/s during application. The application rate was verified.

Sampling. Samples were taken from the control plot one day prior to application and on the day of application. Samples from the treated plot immediately after application and on days 1, 2, 3, 7, 14, 30, 58, 120, 181 and 358 days after application. Sampling with a 5 cm diameter corer, 30 cm depth, 20 cores per sampling date. Cores were deep-frozen and cut into 10 cm layers, corresponding depths were pooled and homogenised with dry ice. Samples were stored deep-frozen for up to 12 months prior to analysis.

Analysis. Soil samples were analysed according to method RAM 475/01. Soil is extracted with acetonitrile:100 mM ammonium acetate acid solution (80:20, v/v) using mechanical agitation. After centrifugation, an aliquot of the soil extract is diluted with acetonitrile/water solution (20:80, v/v) prior to analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS). The limit of quantification of the method is 0.0005 mg/kg.

Reference substances: emamectin benzoate B_{1a}, emamectin benzoate B_{1b}, 8,9-Z-MAB_{1a}, and 8a-OH MAB_{1a}. Concurrent (mean) recovery was 83-92 % at 0.0005 and 0.001 mg/kg for all substances.

Calculations. Dissipation rate constants were calculated using both simple first order (SFO) and first order multi compartment (FOMC) models.

Results

Weather data. Weather data are summarised in the following table.

Table 4.1.4.17-1. Weather conditions during the study

	T [°C] min/max/avg	**Rainfall [mm]**
May 2004[&]	7.1 / 28.0 / 17.6	0.4
June 2004	9.5 / 35.1 / 22.3	6.6
July 2004	11.5 / 35.5 / 23.5	0.8
Aug 2004	11.0 / 35.3 / 23.2	88.4
Sep 2004	7.8 / 34.2 / 21.0	81
Oct 2004	3.1 / 27.2 / 15.2	84.6
Nov 2004	-3.2 / 21.7 / 9.3	46
Dec 2004	-3.3 / 17.3 / 7.0	84
Jan 2005	-6.5 / 17.4 / 5.5	1.8
Feb 2005	-8.5 / 16.3 / 3.9	1.8
March 2005	-9.4 / 21.1 / 5.9	4.8
April 2005	3.2 / 24.9 / 14.1	35.2
May 2005[&]	6.1 / 26.3 / 16.2	38.2

[&] weather data available only for part of the month

No irrigation took take place during the study.

Residues.

No residues of MAB_{1a} and the other analytes were found at any test site prior to the application treatment. Deposition tray soil analysis on basis of MAB_{1a} residues showed a mean recovery of 59% of the expected residues after treatment.

Table 4.1.4.17-1: Theoretical and measured initial concentration of Emamectin benzoate 05 SG in the deposition tray soil samples (n = 5)

Test site	Application rate (g ai/ha)	Theoretical rate of MAB_{1a}[&] (g/ha)	Initial measured residue – mean (range) (g/ha)	Percentage related to theoretical value – mean (range)
Grisolles (F)	22.5	20.25	12.8 (8 – 17)	59 (37 – 79)

Since for none of the analytes residues >LOQ were found in the 10-20 cm layer, samples of the 20-30 cm layer were not analysed at all.

The residue values of MAB_{1a} and MAB_{1b} in the top 0-10 cm layer are summarised in Table. 4.1.4.17-2.

Table. 4.1.4.17-2. Residue levels (mg ai/kg soil) of MAB_{1a} and MAB_{1b} in the 0-10 cm soil layer (wet weight)

Days	Clay loam	
	MAB1a	MAB1b
-4 to 0 (PA)	<LOQ	<LOQ
0	0.0113	0.0008
1	0.0012	<LOQ
2	0.0010	<LOQ
3	0.0008	<LOQ
4	n.s.	n.s.
7	<LOQ	<LOQ
14	0.0006	<LOQ
15	n.s.	n.s.

21	n.s.	n.s.
28	n.s.	n.s.
30	<LOQ	<LOQ
58	<LOQ	<LOQ
59	n.s.	n.s.
60	n.s.	n.s.
112	n.s.	n.s.
120	<LOQ	<LOQ
171	n.s.	n.s.
174	n.s.	n.s.
177	n.s.	n.s.
181	<LOQ	<LOQ
350	n.s.	n.s.
358	<LOQ	<LOQ

n.s. = not sampled

No residues of 8,9-Z-MAB_{1a}, and 8a-OH $MAB_{1a,}$ above the limit of quantification (0.0005 mg/kg) were determined in any soil horizon at any analysis interval during the study.

DT_{50} values for emamectin benzoate B_{1a} reported by the author are summarised in the following table.

Table. 4.1.4.17-3. emamectin benzoate B_{1a} DT_{50} values in silty clay loam soil.

Test site	Simple first order (SFO) model				First order multi compartment (FOMC) model			
	DT_{50} (d)	DT_{90} (d)	r^2	χ^2	DT_{50} (d)	DT_{90} (d)	r^2	χ^2
Grisolles (F)	0.335 RMS: 0.3	1.11 RMS: 0.1	0.983 nc	nr 19.6	0.013 RMS: 0.3	1.13 RMS: 1.2	0.999 nc	nr 19.9

nc: not calculated
nr: not reported

Remarks

RMS recalculated the DT50 for emamectin benzoate B1a, according to SFO kinetics using data of the author. FOMC calculations as provided by the author could not be reproduced. RMS values are included in table –12. The result $DT_{50,field}$ 0.3 days for emamectin benzoate B_{1a} is acceptable.

4.1.4.17 Study 18- Field dissipation

Study reference: Seville, A.G. (2006b), STUDY IIA, 7.3.1/004

Detailed study summary and results

Reference/notifier	:	Seville, A.G. (2006b)	GLP statement	:	yes
Type of study	:	field dissipation, final report	Guideline	:	not specified
Year of execution	:	2004-2005	Acceptability	:	acceptable
Test substance	:	A10324A, a 50 g/kg SG formulation, containing emamectin benzoate, 4.89%			

Substance	Location	Soil type	Land use	Dose [g as/ha]	Date of application	OM [%]	pH&	Duration [d]	$DT_{50,field}$ emamectin benzoate B_{1a} [d]
A10324A	Wallersdorf-See	silty clay loam	bare soil	22.5	07.06.2004	1.9	6.6	350	0.6

& in 0.01M $CaCl_2$

Description

Location. Wallerdorfsee (Germany), silty clay loam, CEC 150 mmol/kg. Bare soil. For a period of up to 3 years prior to the trial, different arable crops were grown on the test site. During the test period Touchdown Quattro was used twice at 5.0 L/ha to keep bare soil conditions.

Application and plot maintenance. The treated area comprised two strips (3 m width, 20 m length) separated by a 6 m wide untreated strip. Each of the treated strips was divided into 2 subplots of 1.5 m width resulting in a total of 4 sub-plots, each 1.5 m wide and 20 m long. In addition, a separate untreated plot (60 m^2) was used for the control samplings. All plots were maintained un-cropped during the test period. Test substance was applied using a wheeled plot sprayer on the 7th of June 2004 in a single treatment of 0.45 kg product/ha in water at 500 L/ha, equivalent to 22.5 g as/ha Weather conditions at time of application: precipitation 0.5 mm within 2 hours after treatment, air temperature 14-15 °C, wind speed 1.5-3.0. The application rate was verified.

Sampling. Control (untreated) samples were taken 0 to 4 days prior to application, and samples for residue analysis were taken on day 0, 1, 2, 4, 7, 14, 21, 28, 60, 112, 171 and 350 after application. . Sampling with a 5 cm diameter corer, 30 cm depth, 20 cores per sampling date. Cores were deep frozen and cut into 10 cm layers, corresponding depths were pooled and homogenised with dry ice. Samples were stored deep frozen for up to 20 months prior to analysis.

Analysis. Soil samples were analysed according to method RAM 475/01. Soil is extracted with acetonitrile:100 mM ammonium acetate acid solution (80:20, v/v) using mechanical agitation. After centrifugation, an aliquot of the soil extract is diluted with acetonitrile/water solution (20:80, v/v) prior to analysis by high performance liquid chromatography with triple quadrupole mass spectrometry detection (LC-MS/MS). The limit of quantification of the method is 0.0005 mg/kg for emamectin benzoate B_{1a}, emamectin benzoate B_{1b}, 8,9-Z-MAB_{1a} and 8a-OH MAB_{1a}.

Reference substances: emamectin benzoate B_{1a}, emamectin benzoate B_{1b}, 8,9-Z-MAB_{1a}, and 8a-OH MAB_{1a}, MFB_{1a} and N-nitroso MAB_{1a}. Mean recovery 88-101 % at 0.0005 and 0.001 mg/kg for all reference substances.

Calculations. Dissipation rate constants were calculated using both simple first order (SFO) and first order multi compartment (FOMC) models.

Results

Weather data. Weather data are summarised in the following table.

Table 4.1.4.18-1. Weather conditions during the study

Time period	T [°C] min/max/avg	Rainfall [mm]
May-2004	6.7 / 17.8 / 12.4	49.2
June-2004	10.5 / 21.5 / 15.8	118.5
July-2004	12.3 / 23.6 / 17.7	102.9
Aug-04	12.8 / 24.8 / 18.8	72.9
Sep-04	8.7 / 19.9 / 14.2	87.4
Oct-2004[&&]	6.0 / 14.5 / 10.1	32.4
Nov-04	1.0 / 5.8 / 3.3	51.8
Dec-04	-3.2 / 0.3 / -1.3	34.1
Jan-2005	-3.7 / 2.5 / -0.5	65
Febr-2005	-6.8 / -0.1 / -3.2	95.5
March-2005	-3.0 / 7.2 / 2.0	32.1
Apr-05	4.6 / 14.9 / 9.9	116.4

May-2005	8.2 / 19.3 / 13.8	103.6

[&] weather data available only for part of the month
[&&] no data available for 9. to 18.10

No irrigation took take place during the study.

Residues.

Since for none of the analytes residues >LOQ were found in the 10-20 cm layer, samples of the 20-30 cm layer were not analysed at all. The residue values of MAB_{1a} and MAB_{1b} in the top 0-10 cm layer of all test sites are summarised in Table. 4.1.4.18-2.

Table. 4.1.4.18-2. Residue levels (mg ai/kg soil) of MAB_{1a} and MAB_{1b} in the 0-10 cm soil layer (wet weight)

Days	Wallersdorf-See (D)	Wallersdorf-See (D)
	MAB_{1a}	MAB_{1b}
-4 to 0 (PA)	<LOQ	<LOQ
0	0.0054	<LOQ
1	0.0015	<LOQ
2	0.0007	<LOQ
3	n.s.	n.s.
4	<LOQ	<LOQ
7	<LOQ	<LOQ
14	<LOQ	<LOQ
15	n.s.	n.s.
21	<LOQ	<LOQ
28	<LOQ	<LOQ
30	n.s.	n.s.
58	n.s.	n.s.
59	n.s.	n.s.
60	<LOQ	<LOQ
112	<LOQ	<LOQ
120	n.s.	n.s.
171	<LOQ	<LOQ
174	n.s.	n.s.
177	n.s.	n.s.
181	n.s.	n.s.
350	<LOQ	<LOQ
358	n.s.	n.s.

n.s. = not sampled

No residues of 8,9-Z-MAB_{1a}, and 8a-OH $MAB_{1a,}$ above the limit of quantification (0.0005 mg/kg) were determined in any soil horizon at any analysis interval during the study.

DT_{50} values for emamectin benzoate B_{1a} reported by the author are summarised in the following table.

Table. 4.1.4.18-3. emamectin benzoate B_{1a} DT_{50} values in silty clay loam soil.

Test site	Simple first order (SFO) model				First order multi compartment (FOMC) model			
	DT_{50} (d)	DT_{90} (d)	r^2	χ^2	DT_{50} (d)	DT_{90} (d)	r^2	χ^2
Wallersdorf-See (D)	N/A RMS:0.6	1.87 RMS:2	0.9958 n.c.	n.r. 10.4	N/A RMS: 1	2.22 RMS: 3.4	0.9995 n.c.	n.r. 28.6

nc: not calculated
nr: not reported

Remarks

DT_{50} for emamectin benzoate B_{1a} is recalculated by RMS according to SFO kinetics, using data of the author. The result $DT_{50,field}$ 0.6 days for emamectin benzoate B_{1a} is acceptable.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

Study reference: STUDY IIA 8.2.6.1/01

Detailed study summary and results

Type of study	:	fish, bioconcentration	GLP statement	:	yes
Year of execution	:	1992 - 1993	Guideline	:	US EPA 54019-82-021; ASTM E1022-84
Test substance	:	MK-244 (emamectin benzoate), batch nr. L-656748-05-2S002, purity 97.5%, appearance off-white crystalline powder. ^{3}H-MAB1a; batch nr. [^{3}H] L-683,825-001A008, ([5-3H]-epimethylaminoavermectin B1a); purity not given, appearance clear liquid	Acceptability	:	acceptable

Substance	Species	Duration [d]	Method	BCF [L/kg wwt]	Based on
^{3}H-MAB1a	*Lepomis macrochirus*	28 uptake 14 depuration	flow-through	82 30 102	total ^{3}H in whole fish total ^{3}H in fillet total ^{3}H in viscera

Materials and methods

Description

Methods. Bluegill sunfish were exposed to emamectin benzoate and ^{3}H-MAB1a for 28 days to measure uptake of the compound and then placed in clean water for 14 days to determine elimination rate. Fish were commercially obtained, and acclimated for at least 14 days, mean weight 1.48 g and mean length 45.9 mm at test initiation. Experiment was performed in two phases in which the second phase mimicked the first phase and served to generate tissue samples to evaluate the identity and distribution of MK-244 and its metabolites in fish. Flow-through system, continuous aeration, filtered well water hardness 141 mg $CaCO_3$/L and conductivity 333 µmhos/cm in phase I and 328 mg $CaCO_3$/L and 328 µmhos/cm during phase 2. 30 L test solution per system. The test solutions were renewed to produce 9.6 volume additions per 24 hours. Prior to initiating the uptake phase of the study, the test solutions were allowed to flow through the test chambers for a 3-5-day equilibrium phase. Two replicate test chambers in both the treatment and control group (solvent methanol) with 90 fish per chamber (92 fish in phase 2). One exposure level at 1.6 µg/L. Daily observations.

Environmental conditions. Temperature 21.7 to 22.3 ºC, pH 7.7 to 8.2. Mild aeration was initiated in all test chambers on day 15 to ensure that oxygen concentrations remained ≥ 60 %.

Chemical analysis. Water and fish were sampled throughout the uptake and depuration period. Fish samples were taken for tissue analysis at 1, 3, 7, 10, 14, 21 and 28 days into the uptake phase and 1, 3, 7, 10 and 14 days into the depuration phase. On each of the fish sampling occasions, 2 fish were randomly selected from each test vessel. Total radioactive residues in the non edibles (internal organs, carcass, fins) and edible (flesh) tissues were analysed by combustion in a sample oxidiser. The values obtained were added together to calculate the whole body total radioactive residue. Tissues were then extracted with a mixture of organic solvents for subsequent determination of residue profiles by RP- and NP-HPLC-radio-chromatographic

analyses. The analytical methods employed to measure the concentrations of in the test solutions were based on LSC to determine total [^{3}H] residues. On day 28 of the uptake period, duplicate water samples were collected to evaluate the presence of any ^{3}H-MAB1a or degradates. Samples of approximately 1000 mL were taken from all test chambers.

LOQ 2.5 µg as equivalents/kg for fish samples and LOQ 0.050 µg as equivalents/L for water samples. Recovery 80 % for edible fish samples, 78% for non edible fish tissue.

Calculations and statistics. Steady-state concentration was considered to be achieved when three consecutive sets of tissue samples were not statistically different ($p > 0.05$) 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.

Results

Mortality <1% in control and treatment and no abnormal behaviour. The mean measured radioactivity concentration in the treatment chambers during the exposure phase, was equivalent to 1.2 ± 0.095 µg ^{3}H-MAB1a/L.

For confirmation that the radioactivity present was ^{3}H-MAB1a, the test item was monitored after 28 days of exposure by means of solid phase extraction and HPLC-analysis. It was found that ^{3}H-MAB1a was stable in water under the ambient flow through conditions of the study.

The concentrations of ^{3}H-MAB1a in fish tissue during the 28 day uptake phase followed by the 8 day depuration phase for fathead minnow are given in the table below.

Table 4.2-1 Water and tissue concentrations and corresponding BCF-values during uptake and depuration of 3H-MAB1a in the fish tissues.

	Mean concentration of ^{3}H-MAB1a						
	Day of study	**Water**	**Edible Tissues**		**Non Edible Tissues**		**Whole Fish**	
		(µg/L)	**(µg/kg)**	**BCF**	**(µg/kg)**	**BCF**	**(µg/kg)**	**BCF**
Uptake phase	**0**	1.2	> LOQ	n.a.	> LOQ	n.a.	> LOQ	n.a.
	1	1.2	6.5	5.4	32	27	21	18
	3	1.1	16	15	67	61	43	39
	7	1.2	22	18	98	82	66	55
	10	1.4	30	21	117	84	80	57
	14	1.3	28	22	110	85	76	58
	21	1.3	37	28	136	105	94	72
	28	1.3	40	31	128	98	90	69
Depuration phase	**1**	< LOQ	35	n.a.	128	n.a.	90	n.a.
	3	< LOQ	23	n.a.	87	n.a.	60	n.a.
	7	< LOQ	7.5	n.a.	33	n.a.	22	n.a.
	10	< LOQ	6.7	n.a.	31	n.a.	19	n.a.
	14	< LOQ	2.9	n.a.	14	n.a.	9.0	n.a.

LOQ for water samples: 0.05 µg/L; LOQ for tissue samples: 2.5 µg/kg.
n.a.: not applicable.

According to the author, the radioactivity, expressed as ^{3}H-MAB$_{1a}$ equivalents, was found to accumulate within the tissues and reached a plateau concentration between day 7 and day 14. The steady-state in edible tissue was however not reached. Consequently, the BCF value for edible tissue was estimated based on the

day 28 tissue concentration. The BCF values for nonedible tissue and whole fish were calculated based on the mean tissue concentration of days 7, 10 and 14. The observed bioconcentration factors for edible tissue, nonedible tissue and whole fish were reported as 33, 90 and 62, respectively. The uptake rate constant (K_1) for ^{3}H-MAB_{1a} was calculated to be 14 ml/g/day/day for whole fish tissues. The depuration rate constant (K_2) was calculated to be 0.18/day. The kinetic bioconcentration factors (BCF_K) for edible tissue, nonedible tissue and whole fish were calculated to be 30, 116 and 80.

The total residues in edible tissues averaged about 23 mg/kg and about 106 mg/kg in non-edible issues. Based on coincidence in retention time, the presence of parent ^{3}H-MAB_{1a} and its demethylated product AB_{1a} was determined. The levels for ^{3}H-MAB_{1a} and AB_{1a} in the edible tissues averaged about 12 and 3 mg/kg, accounting for 54 and 12%, respectively, of total radioactive residues. In non-edible tissues the levels averaged to 53 and 10 mg/kg, accounting to 49 and 9% respectively. In both fractions, low level radioactive residues were determined, totalling about 34 and 38% in edible and non-edibles tissues respectively. However, none of these residues came over 1 µg/kg or 5% of the total radioactive residues.

Remarks

Test criteria were met. Bioconcentration factors were miscalculated by the author. For edible tissue the BCF at the chosen plateau of 28 days is 31 and the mean BCF for nonedible tissue and whole fish as determined over days 7, 10 and 14 the BCFs should be 84 and 57 (was however calculated over days 7, 10, 14, 21 and 28). RMS considers the plateau BCF-values of nonedible tissue and whole tissue to be higher than those determined by the author. As higher values were attained after 14 days it is not correct to consider the period between 7 and 14 days to be a plateau.

According to OECD guideline 205 the test substance in fish taken at three consecutive analyses which are taken at at least two days intervals, should be within 20% of each other. Also, there should not be significant differences among the three sampling periods.

Plateau levels are considered to be between 21 and 28 days by RMS. The experiment has not lasted long enough to reach three consecutive samples in the steady state. The timepoints of the analyses have not been chosen well. If one extra timepoint had been chosen in between 21 and 28 days, the plateau could have been confirmed. For the 21 – 28 days interval, the recalculated mean BCFs are 30, 102 and 82 for edible tissue, nonedible tissue and whole fish, respectively. BCFs are given in the table below.

Table 4.2-2 BCF-values during uptake of 3H-MAB1a in fish tissues

	BCF edible tissue	**BCF nonedible tissue**	**BCF whole fish**
reported BCF	33	90	62
recalculated with same plateau	31	84	57
recalculated with plateau chosen by RMS	**30**	**102**	**82**
BCF (K1/K2)	30	116	80
recalculated BCF (K1/K2)	31	111	78

As the BCF-values determined by RMS closely match the recalculated BCF (K1/K2), the steady state period as determined by RMS is considered to be correct. Residues could be identified to be ^{3}H-MAB1a and its demethylated product AB1a predominantly. Unidentified low level radioactive residues were determined as well, however, none of these residues came over 1 µg/kg or 5% of the total radioactive residues.

BCF based on total radioactivity, transformation may have taken place and values are *worst case.* The results BCFs of 30, 102 and 82 L/kg wwt for fillets, viscera and whole fish respectively, are acceptable. Residues are emamectin benzoate and its demethylated product AB_{1a} predominantly.

4.2.2 Bioaccumulation test with other organisms

No data available.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

4.3.1.1 Study 1 - acute toxicity fish

Study reference: STUDY IIA 8.2.1.1/01

Detailed study summary and results

Type of study	:	fish, acute toxicity	GLP statement	:	yes
Year of execution	:	1992	Guideline	:	ASTM E 729-88; EPA 540/9-82-024
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S002, purity 95.9 %, appearance white powder	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
emamectin benzoate	*Oncorhynchus mykiss*	flow-through	11.2-12.2	8.2-8.3	96	LC_{50}	174

Description

Methods. Toxicity of MK-244 to rainbow trout (*Oncorhynchus mykiss*) was tested under flow-through conditions. Trout eggs were purchased from a commercial supplier and were hatched and held in the culture facility of the performing laboratory. Fish were acclimated for 48 hours to test conditions, length 44 - 53 mm and mean wet weight 1.76 – 2.90 g at start. Nominal concentrations 26, 43, 72, 120 and 200 µg/L as pure compound, control, solvent control (0.1 mL methanol/L). Dilution with natural well water passed through a sand filter, total hardness 140 - 152 mg $CaCO_3$/L, conductivity 320 – 335 µmhos/cm, pH 8.2 – 8.3. Two replicates with 10 fish each, 15 L water per test vessel, renewal rate 7.5 L/h. Observations 4, 24, 48, 72 and 96 h after test initiation.

Conditions. Temperature 11.2 – 12.2 °C, 16:8 h L:D (861 lux, with 30 minutes transition period), no aeration, no feeding.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis by both HPLC-fluorescence (three lowest concentrations) and UV detection methods (two highest concentrations). HPLC-UV after extraction with ethyl acetate, LOQ 0.1 µg/L. Mean procedural recovery of fluorescence detection analysis was 104%. Mean procedural recovery of UV detection analysis was 70%.

Calculations and statistics. LC_{50}-values were determined using the program of Stefan (1977) and visual interpretation. NOEC was determined by visual interpretation of mortality data.

Results

Measured concentrations were corrected for mean procedural recovery. Actual concentrations were 67 - 124 % of nominal at start and 62 - 142 % of nominal at end. Mean measured concentrations were 21.1, 30.4, 48.7, 132 and 246 µg/L (68 - 123 % of nominal). No mortality in controls and solvent controls, and at 21.1 – 48.7 µg/L, 20 % mortality at 132 µg/L, 85 % at 246 µg/L, first deaths after 96 hours. 96-hours LC_{50} reported

as 174 µg/L (95 % CL 146 – 207 µg/L). LC_{50}-values for the 96 h exposure period was calculated using binomial probability and the LC_{50} for 24, 48 and 72 hours of exposure was based on visual interpretation.

Remarks

Water quality parameters within accepted limits. The result 96-hours LC_{50} 174 µg/L, based on mean measured concentrations, is.

4.3.1.2 Study 2 - acute toxicity fish

Study reference: STUDY IIA 8.2.1.2/01

Detailed study summary and results

Type of study	:	fish, acute toxicity	GLP statement	:	yes
Year of execution	:	1992	Guideline	:	ASTM E 729-88; EPA 540/9-82-024
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S002, purity 95.9 %, appearance white powder	Acceptability	:	acceptable

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[µg/L]
emamectin benzoate	*Lepomis macrochirus*	flow-through	21.2 – 21.6	8.1 – 8.2	96	LC_{50}	180

Description

Methods. Toxicity of MK-244 to bluegill was tested under semi-static test conditions. Juveniles were commercially obtained, held under test conditions for 14 days and acclimated for 2 days, length 26 mm and weight 0.42 g at start. Nominal concentrations 0.05, 0.09, 0.14, 0.24 and 0.40 mg/L, control, solvent control (0.10 mL methanol/L). Dilution with well water that was passed through a sand filter, hardness 156 mg $CaCO_3$/L, pH 8.0, conductivity 342 µmhos/cm. Daily renewal rate 7.5 L/h. Two replicates with 10 fish, 15 L water. Observations for mortality and abnormal behaviour were made 2, 24, 48, 72 and 96 h after initiation of the test.

Conditions. Temperature 16:8 h L:D (861 lux, with 30 minutes transition period), aeration, no feeding.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis with UV detection. Mean procedural recovery of UV detection analysis was 80% with LOQ 0.05 mg/L.

Calculations and statistics. LC_{50}-values were determined using the program of Stefan (1977) using probit analysis (at 48 and 72 h) or binomial probability (at 24 and 96 h) with nonlinear interpolation. NOEC was determined by visual interpretation of mortality data.

Results

Actual concentrations were 39 - 67 % of nominal in fresh solutions, and 75 - 98 % of nominal in old solutions. In between actual concentrations were 78 – 158 % of nominal. Mean measured concentrations were 0.056, 0.087, 0.14, 0.24 and 0.35 mg/L (88 - 112 % of nominal). Actual concentrations were considerably below nominal at the start of the experiment, but approximated nominal values at later sampling points. This was not considered to affect the outcome of the toxicity test.

No mortality in control, solvent control, and the two lowest concentrations, 5 % mortality at 0.14 mg/L, 100 % at 0.24 and 0.35 mg/L by day 4. 96-hours LC_{50} reported as 0.18 mg/L (95 % CL 0.14 – 0.24 mg/L, 96-hours NOEC was 0.087 mg/L, all based on mean measured concentrations.

Remarks

Water quality parameters within accepted limits. The result 96-hours LC_{50} 180 µg/L, based on mean measured concentrations,.

4.3.1.3 Study 3 - acute toxicity fish

Study reference: STUDY IIA 8.2.1.2/02

Detailed study summary and results

Type of study	:	fish, acute toxicity	GLP statement	:	yes
Year of execution	:	1992	Guideline	:	ASTM E 729-88; EPA 540/9-82-024
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 94.6 %, appearance white powder Radiolabelled MK-244, batch L-683,825-055J006, 15994-111/95-137, purity 99.3%	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
^{3}H-emamectin MAB_{1a} and emamectin benzoate	*Pimephales promelas*	flow-through	21.5-22.5	8.3-8.4	96	LC_{50}	194

Description

Methods. Toxicity of MK-244 to fathead minnow (*Pimephales promelas*) was tested under flow-through conditions. Eggs were purchased from a commercial supplier and were hatched and held in the culture facility of the performing laboratory. Fish were held for 77 days prior to testing and acclimated for 52 hours to test conditions, length of control fish 24 – 29 mm and mean wet weight 0.20 – 0.38 g at end of test. Loading 0.19 g/L. Nominal concentrations 39, 65, 108, 180 and 300 µg/L as pure compound, control, solvent control (0.1 mL methanol/L). Dilution with natural well water passed through a sand filter, total hardness 132 mg $CaCO_3$/L, conductivity 328 µmhos/cm, pH 8.2 – 8.3. Two replicates with 10 fish each, 15 L water per test vessel, renewal rate 7.5 L/h. Observations 2.5, 24, 48, 72 and 96 h after test initiation.

Conditions. 16:8 h L:D (475 lux, with 30 minutes transition period), no aeration, no feeding.

Chemical analysis. Samples were collected at beginning and at end of test. Analysis by LSC. Samples of stock solution were analysed by LSC followed by HPLC, LOD 0.025 µg/L. Recovery in HPLC analysis was 99 – 102% of nominal concentrations.

Calculations and statistics. LC_{50}-values were determined using the program of Stefan (1977) and visual interpretation. NOEC was determined by visual interpretation of mortality data.

Results

Measured concentrations were corrected for mean procedural recovery. Actual concentrations were 74 - 88 % of nominal at start and 67 - 87 % of nominal at end. Mean measured concentrations were 27, 48, 89, 156 and 257 µg/L (69 -87 % of nominal). No mortality in controls and solvent controls, and at 27 – 89 µg/L, 5 % mortality at 156 µg/L, 100 % at 257 µg/L, first deaths after 72 hours. 96-hours LC_{50} reported as 194 µg/L (95

% CL 156 – 257 µg/L). LC_{50}-values for the 96 h exposure period was calculated using binomial probability and the LC_{50} for 24, 48 and 72 hours of exposure was based on visual interpretation.

Remarks

Water quality parameters within accepted limits. The result 96-hours LC_{50} 194 µg/L, based on mean measured concentrations.

4.3.1.4 Study 4 acute toxicity fish

Study reference: STUDY IIA 8.2.1.2/04

Detailed study summary and results

Type of study	:	fish, acute toxicity	GLP statement	:	yes
Year of execution	:	1994	Guideline	:	ASTM E 729-88; EPA 540/9-82-024
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 95.9 %, appearance white powder	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
emamectin benzoate	*Cyprinodon variegatus*	flow-through	21.9-22.3	8.0-8.2	96	LC_{50}	1430

Description

Methods. Toxicity of emamectin benzoate to sheepshead minnow (*Cyprinodon variegates)* was tested under flow-through conditions. Fish were reared in own laboratory facilities. Fish were held under test conditions for 14 days and acclimatised for 50 h prior to testing, length of control fish 26 – 31 mm and mean wet weight 0.49– 0.98 g at end of test. Loading 0.47 g/L. Nominal concentrations 0.35, 0.58, 0.97, 1.62 and 2.70 mg/L as pure compound, control, solvent control (0.10 mL methanol/L). Dilution with natural sea water which was passed through a sand filter and diluted with well water to a salinity between 24 and 25 $^{o}/_{oo}$, Two replicates with 10 fish each, 15 L water per test vessel, renewal rate 7.5 L/h. Observations 3, 24, 48, 72 and 96 h after test initiation.

Conditions. 16:8 h L:D (323 lux, with 30 minutes transition period), no aeration, no feeding 48 h prior to testing and during the test.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis by HPLC with UV detector, LOQ 0.2 mg/L. Direct injection apportion of the study sample. Mean procedural recovery of was 113%, 140% and 123% at fortifications of 0.20, 1.0 and 4.0 mg as/L. Overal procedural recovery was 124%.

Calculations and statistics. LC_{50}-values were determined using the program of Stefan (1977) using the probit analysis and visual interpretation.

Results

Measured concentrations were corrected for mean procedural recovery. Actual concentrations were 85 - 109 % of nominal at start and 83 - 101 % of nominal at end. Mean measured concentrations were 0.33, 0.50, 0.86, 1.43 and 2.63 mg/L. No mortality in controls and solvent controls, and at 0.33, 0.50 amd 0.86 mg/L, 60 % mortality at 1.43 mg/L, 95 % at 2.63 mg/L, first deaths after 72 hours. Sublethal effects (discolouration) at 0.50 mg/L. 24-hours LC_{50} > 2.63 mg/L, 48-hours LC_{50} >2.63 mg/L, 72-hours LC_{50} >2.63 mg/L and 96-hours 1.43 mg/L (95 % CL 1.25 – 1.67 mg/L). LC_{50}-values for the 96 h exposure period was calculated using probit analysis and the LC_{50} for 24, 48 and 72 hours of exposure was based on visual interpretation.

Remarks

Water quality parameters within accepted limits. The result 96-hours LC_{50} 1430 µg/L, based on mean measured concentrations.

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 Study 1 - oyster embryo, acute toxicity

Study reference: STUDY IIA 8.3.1.1/03

Detailed study summary and results

Type of study	:	oyster embryo, acute toxicity	GLP statement	:	yes
Year of execution	:	1983	Guideline	:	ASTM E 729-888, EPA 540/9-82-024
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 95.9 %, appearance white powder	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
emamectin benzoate	*Crassostrea virginica*	flow-through	22.0-22.1	7.9-8.1	96	EC_{50}	530

Description

Methods. Effects of emamectin benzoate on deposition of the eastern oyster, *Crassostrea virginica*, was tested under flow-through conditions. Oysters were obtained from a commercial company. Oysters were held under test conditions for at least 10 days prior to test initiation. Length of the oysters was 27 to 42 mm. Nominal concentrations 0.10, 0.17, 0.29, 0.48 and 0.80 mg/L, control, solvent control (methanol 0.10 mL/L). Dilution with filtered natural seawater, salinity 25 ‰. Daily observations for clinical signs of toxicity and mortality. At test termination the mean shell deposition was calculated by measuring the longest finger of growth for each oyster with calipers. Shell deposition was expressed as percent inhibition of mean shell growth for each treatment relative to control shell growth. One replicate with 20 oysters each, 13 L water per test vessel, renewal rate 20 L/h.

Conditions. 16:8 h L:D (30 min. transition period) 258 lux, no aeration, feeding with an algal suspension during holding and throughout the test.

Chemical analysis. Samples were collected at beginning and at 24 h intervals. Analysis by HPLC with UV detector, LOQ 80 µg/L. Overal procedural recovery was 95%.

Calculations and statistics. EC_{50}-value was calculated according to Stephan (1977) using probit method. The observed effect concentration was determined by visual inspection of the data.

Results

Measured concentrations were corrected for mean procedural recovery. Actual concentrations were 76 - 120 % of nominal at start and 84 - 110 % of nominal at end. Mean measured concentrations were 0.10, 0.15, 0.26, 0.42 and 0.77 mg/L. Shell deposition was 3.03 mm in the pooled controls. Percent inhibition was -7.3, -9.6, -29, 47 and 72% at 0.10, 0.15, 0.26, 0.42, 0.77 mg as/L. 96-LC_{50} reported as 0.53 mg as/L (95 % CL 0.35 – 1.2 mg/L).

Remarks

Water quality parameters within accepted range. The highest three test concentrations above water solubility in freshwater (0.24 mg/L at pH 7), but the solvent control was used to increase solubility. No flocculation reported for these concentrations. The result EC_{50} 530 µg/L, based on mean measured concentrations.

4.3.2.2 Study 2 - mysid shrimp, acute toxicity

Study reference: STUDY IIA 8.3.1.1/04

Detailed study summary and results

Type of study	:	mysid shrimp, acute toxicity	GLP statement	:	yes
Year of execution	:	1993	Guideline	:	EPA 540/9-82-024, EPA540/9-85-010, ASTM E 729-88
Test substance	:	MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 95.9%, appearance white powder; ^{3}H-MAB1a (emamectin B1a), batch L683,825-001A009; 18075-148; 93-014, radiochemical purity 97.2% (TLC, scanning) appearance clear solution	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
^{3}H-emamectin MAB_{1a} and emamectin benzoate	*Mysidopsis bahia*	flow-through	24.6-25.0	8.2-8.3	96	LC_{50}	0.040

Description

Methods. Acute toxicity of ^{3}H-MAB_{1a} to juvenile *Mysidopsis bahia* reared at Wildlife International Ltd., was tested under flow-through conditions. Nominal concentrations 13, 22, 36, 60 and 100 ng/L, control, solvent control (methanol, 0.1 mL/L). Dilution with filtered natural seawater (5 µm mesh), salinity 20 ‰, pH 7.7. Two replicates with 10 organisms each, 6.5 L solution per unit, flow-rate 28 volume additions of test water per day. Observation on mortality, signs of toxicity and abnormal behaviour were performed 6, 24, 48, 72 and 96 h after test initiation.

Conditions. 16:8 h L:D (215 lux), no aeration, daily feeding with live brine shrimp.

Chemical analysis. Daily samples to determine actual concentrations. Analysis by LSC, LOQ was set at 58.92 (2.2 ng/L) based on twice the dpm found for the liquid scintillant background. For analysis with HPLC-UV samples were evaporated to dryness and reconstituted in acetonitrile. LOQ 0.10 mg/L. Mean procedural recovery 94%.

Calculations and statistics. 72 and 96-h LC_{50}-values and 95% confidence limits were calculated using probit method of calculation according to Stefan (1977).

Results

Mean measured concentrations 7.8, 18, 26, 41 and 72 ng/L. At day 0 recovery ranged from 54 to 71 % of nominal values. At 96 h recovery ranged from 65 to 85% of nominal. Measured concentrations at test initiation were always lower than concentrations measured later during the test. The test substance was stable during the experiment. No control mortality and no mortality in solvent control and at 7.8 and 18 ng/L. 10, 45 and 100 % mortality at 26, 41 and 72 ng/L. LC_{50} for 96 hours exposure reported as 0.040 µg/L (95 % CL 0.035 – 0.046 µg/L), based on mean measured concentrations.

Remarks

Water quality parameters within accepted range. The result 96-h LC_{50} 0.040 µg/L, based on mean measured concentrations.

4.3.2.3 Study 3 - Daphnia, acute toxicity

Study reference: STUDY IIA 8.3.1.1/01

Detailed study summary and results

Type of study	:	Daphnia, acute toxicity	GLP statement	:	yes
Year of execution	:	1992	Guideline	:	US EPA 540/9-82-024; ASTM E 729-88
Test substance	:	MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 95.9%, appearance white powder	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg/L]
emamectin benzoate	*Daphnia magna*	flow-through	19.8 – 19.9	8.1 – 8.2	48	LC_{50}	1.0

Description

Methods. Daphnids (< 24 h old) exposed to emamectin benzoate for 48 h in flow-through test systems (300 mL glass beakers). Reconstituted deionised water, total hardness 143 mg $CaCO_3$/L, pH 7.8 – 8.1, 320 - 380 µmhos/cm. Nominal concentrations 0.39, 0.65, 1.08, 1.80 and 3.00 µg/L, control, solvent control (methanol, 0.1 mL/L). Two replicates for control and test compound, 10 daphnids per test unit. Dilution with filtered well water, flow-rate 28 volume additions of test water per day. Observations of immobility, mortality and other clinical signs of toxicity were made at 3.5, 24 and 48 h after test initiation

Conditions. no aeration, 16:8 h L:D (30 min transition period, 431 lux), no feeding.

Analytical methods. Samples were collected at 0, 24 and 48 h after test initiation and analysed by HPLC with fluorescence detection method. Extraction with ethyl acetate. After evaporation to dryness the sample was reconstituted with acetonitrile. LOQ 0.10 µg as/L. Derivatization with 1-methylimidazole. Mean procedural recovery 99%.

Calculations and statistics. The 48-h EC_{50}-value and 95% confidence interval was calculated using probit method according to the methods of Stephan (1977). 24-h EC_{50}-value was established by visual inspection of the data.

Results

Recovery at test initiation ranged from 67 to 94% of nominal and at end 63 to 81% of nominal. Mean measured concentrations were 0.30, 0.47, 0.85, 1.38 and 2.26 µg/L (corrected for mean procural recovery of 99 %). No mortality in control, solvent control and two lowest concentrations.15 % at 0.85 µg/L, 45 and 80 % mortality at 1.38 and 2.26 µg as/L. Nominal 48-hours EC_{50} reported as 1.0 µg/L (95% CL 0.84 – 1.19 µg/L).

Remarks

Water quality parameters within accepted range. The 48-h EC_{50} of 1.0 µg/L based immobility and mortality and based on mean measured concentrations.

4.3.3 Algal growth inhibition tests

4.3.3.1 Study 1 - algae, growth inhibition

Study reference: STUDY IIA 8.4/001

Detailed study summary and results

Type of study	: algae, growth inhibition	GLP statement	: yes
Year of execution	: 1995	Guideline	: US EPA 540/9-82-020 nontarget plants
Test substance	: MK-244 (emamectin benzoate), batch L-656, 748-052S002, purity 94.6 %, appearance white powder and ^{3}H-MK-244, batch L-683,825-005J006, appearance clear liquid	Acceptability	: acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg//L]
emamectin benzoate	*Pseudokirchneriella subcapitata*[1]	static	22.8 - 23.5	7.3 – 9.6	120	E_rC_{50}	> 3.9
						NOEC	≥ 3.9

1: formerly known as *Selenastrum capricornutum*

Description

Methods. Static growth inhibition test with active substance emamectin benzoate, nominal concentration 11 µg/L, control and solvent control (dimethylformamide 0.1 mL/L). Three replicates for test substance and control, 100 mL test solution per test unit, initial cell density 3 x 10^3 cells/mL. Cells were counted after 24, 48, 72, 96 and 120 h.

Chemical analysis. Radioactivity was measured with LSC (LOQ 1.8 µg a.s./L) at 0 and 5 d. Concentrations were measured by HPLC with UV detection LOD 6.25 ng. Procedural recovery by LSC and HPLC were both 109 %.

Conditions. Continuous light (3890 – 4300 lux).

Calculations and statistics. Student's t-test was used to determine significant differences between treatment and solvent control.

Results

Actual concentration was 3.86 µg a.s./L at start (35 % of nominal) and 2.75 µg as/L at end (25 % of nominal). Test results were based on initial concentrations. It was suspected that the lipophilic character of the test substance resulted in adsorption of some of the chemical to the algal cells or test flask walls. Exponential growth in the control. Significant differences between control and solvent control. Percent inhibition was calculated in relation with the solvent control. Growth rate (based on log transformed densities) was reduced with 1.6% and cell densities with 9.9%, both reductions were not significant. E_rC_{50} reported as > 3.9 µg a.s./L, based on initial concentration. NOEC for growth weight was ≥ 3.9 µg a.s./L.

Remarks

The pH at 0 and 120 h differed with more than 2 points in the test substance (pH 7.4 – 9.6). Only one concentration was tested and the actual concentration was too low. The experiment was not suitable to determine an accurate EC_{50}. The result a 120-h E_rC_{50} > 3.9 µg a.s./L based on initial concentrations.

4.3.3.2 Study 2 - algae, growth inhibition

Study reference: STUDY IIA 8.4/002

Detailed study summary and results

Type of study	: algae, growth inhibition	GLP statement	: yes
Year of execution	: 2003	Guideline	: US EPA OPPTS 850.5400; OECD 201; EC, L383 A, Part C.3
Test substance	: MK-244 (emamectin benzoate), batch SSH2F004, purity 97.3 %, appearance white powder	Acceptability	: acceptable

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [µg//L]
emamectin benzoate	*Pseudokirchneriella subcapitata*[1]	static	23.9 – 24.1	7-3 -9.7	96	E_rC_{50}	12.1

1: formerly known as *Selenastrum capricornutum*

Description

Methods. Static growth inhibition test with active substance emamectin benzoate, nominal concentrations 0.0031, 0.0063, 0.013, 0.025, 0.050, 0.10, 0.20 and 0.40 mg/L, control with culture medium only. Three replicates for test substance and six replicates for the control, 100 mL test solution per test unit, initial cell density 0.96 x 10^4 cells/mL. Cells were counted after 24, 48, 72 and 96 h.

Chemical analysis. Concentrations were measured by HPLC with UV detection (245 nm) at 0 and 4 d, LOD 1.4 µg/L. Procedural recovery by HPLC was not documented.

Conditions. Continuous light (4330 lux).

Calculations and statistics. Growth rate was determined using log transformed densities. Dunnett's test was used to determine significant differences between treatments and control. A Weibull model was fitted to the percent effect and log concentration data, this model was used to estimate the median effective concentration and its 95% confidence limits.

Results

Measured concentrations at start ranged from 64 – 88% of nominal (>80% at all concentrations >0.050) and at the end from below LOQ (lowest four concentrations) to 38% of nominal. Test results are based on mean measured concentrations (below LOQ – 55% of nominal) <0.0014, <0.0017, <0.0024, <0.0034, <0.0046, 0.012, 0.036, 0.085 and 0.22 mg/L. Exponential growth in the control. 96-h E_bC_{50} reported as 5.99 µg/L (95% CL 5.85 – 6.14 µg/L) and 96-h E_rC_{50} reported as > 220 µg/L. Based on the study report, the NOEC was determined to be <4.6 µg/L.

Remarks

The pH at 0 and 96h differed with more than 2 points at 3 lowest concentrations. The author considered this shift to be the result of high growth factors. Differences were less than 1 point at the 4 highest concentrations. Recalculated 96-h E_bC_{50} was 7.2 µg/L (95% CL 5.4 – 9.5 µg/L) and 96-h E_rC_{50} was 12.1 µg/L (95% CL 10.5 – 13.9 µg/L). Biomass data indicated that there was an artefact as biomass seemed to increase at higher concentrations. Moreover, the growth rate did not decrease to 0. As exponential growth was still present in the control at 96 h the data can be used.The E_rC_{50} calculated by the author seems to be a mistake. The result, a 96-h E_bC_{50} of 7.2 µg/L is considered aceptable.

4.3.4 *Lemna* sp. growth inhibition test

Study reference: STUDY IIA 8.6/01

Detailed study summary and results

Type of study	: Lemna, growth inhibition	GLP statement	: yes

Year of execution	:	1995	Guideline	:	US-EPA 540/9-82-020; ASTM E 1415-91
Test substance	:	MK-244 (emamectin benzoate), batch nr.L656,748-052S005, purity 94.6 %, appearance-white powder and ^{3}H-MK-244 batch nr. L-683,825-005J006, purity 99.3%, appearance a clear liquid	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg/L]
radiolabelled emamectin benzoate	*Lemna gibba*	static-renewal	24.3-24.8	4.9 – 6.2	14	IC_{50}	>94

Description

Test substance: MK-244 (emamectin benzoate), batch nr.L656,748-052S005, purity 94.6 %, appearance-white powder and 3H-MK-244 batch nr. L-683,825-005J006, purity 99.3%, appearance a clear liquid. *Methods*. Toxicity of emamectin benzoate n to *Lemna gibba* was assessed under static-renewal conditions by exposure through the water phase. Nominal test concentrations 11 and 110 µg /L, negative (culture medium) control, solvent control (0.1 mL DMF/L). Test medium was M-type Hoagland's medium without EDTA or sucrose, pH adjusted to 5.0 ± 0.1. Test units contained 100 mL test medium, three replicates for the test treatments and the control and two replicates for the solvent control. 15 fronds and 5 plants per replicate. Test solutions were prepared at days 0, 3, 6, 9 and 12 and were adjusted to 100% purity of the active substance. Number of fronds determined on days 3, 6, 9, 12 and 14. In addition the total number of duckweed plants in each replicate test chamber was determined on day 14. Observations on chlorosis, necrosis, dead fonds, root destruction and break-up of duckweed colonies were made on days 3, 6, 9, 12 and 14. Glassware was silanized prior to use to minimize loss of the test substance through adherence.

Conditions. Continuous light (4630 to 5620 lux).

Chemical analysis. Radioactivity in water and plants was determined with LSC, LOQ 1.7 µg a.s. equivalents/L. HPLC-UV (245 nm) was used to determined concentrations in water (LOD 25 µg/L). Procedural recovery 106 and 105% for 0.11 and 1.1 µg/L, respectively.

Calculations and statistics. Mean plant and frond counts were used to calculate the percent inhibition. A t-test was used to compare negative and solvent control groups. Pooled control replicates were used for further statistical evaluations. Numbers of plants and fronds, percentage of dead fronds, necrotic fronds and chlorotic fronds was evaluated for normality and homogeneity of variances using the Chi-square test and Bartlett's test, respectively. All of the parameters were then analysed statistically using the Bonferroni t-test.

Results

Mean concentrations of fresh solutions were 6.8 and 94 µg/L (62 and 85% of nominal) as determined by LSC. Concentrations in test chambers collected on days 1, 2, 3 and 14 were lower (49% of the mean values for the 110 µg/L solution). Plant tissue concentrations were <LOQ at day 3 and 55-78 µg/kg tissue at day 14. Doubling time of frond number in the controls was almost 3 days. Mean number of fronds after 14 days was 728 in control and 724 in solvent control, and 714 and 721 at 6.8 and 94 µg/L, respectively. Percent inhibition of frond numbers was 1.7 and 0.69%, both not significant. Mean number of plants after 14 days was 232 in control and 217 in solvent control, and 231 and 244 at 6.8 and 94 µg/L, respectively. No inhibition. No significant differences in percentages of dead, chlorotic, or necrotic fronds. IC_{50} (fronds) reported as > 94 µg/L, based on mean measured concentrations.

Remarks

The result an IC_{50} > 94 µg/L, based on mean measured concentrations of fresh solutions is considered acceptable.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

Study reference: STUDY IIA 8.2.4/01

Detailed study summary and results

Type of study	:	fish, early life stage toxicity	GLP statement	:	yes
Year of execution	:	1995	Guideline	:	ASTM E1241-88, 1988 US EPA 540/9-82-024, 1982 and 540/9-86-138, 1986
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S005, purity 94.6 %, appearance white powder and ^{3}H-MK244, batch [3H] L-683,825-005J006, ([5-3H] epimethylaminoavermectin B_{1a} benzoate) and L-683,825-005J006, ([5-3H] epimethylaminoavermectin B_{1a} benzoate), clear liquids, substances suspended in ethanol	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg/L]
^{3}H-epimethylaminoavermectin B_{1a} benzoate	*Pimephales promelas*	flow-through	24.7 – 25.1	8.1 – 8.4	32	NOEC	12

Description

Methods. Early life stages of fathead minnow were exposed to emamectin benzoate under flow-through conditions. Nominal concentrations 3.8, 7.5, 15, 30 and 60 µg/L, control, solvent (methanol). Well water with total hardness 124 -136 $CaCO_3$ mg/L, conductivity 340 - 370 µmhos/cm. Commercially obtained eggs were incubated in oscillating cups placed in flow-through chambers until hatching, two replicates per concentration, each replicate consisting out of two incubation cups containing 20 embryos. Flow-through chambers with 7 L test solution and flow-rate of 3.8 L/h. Daily observation of egg mortality until hatch. Daily observation of larvae for mortality and signs of toxicity or abnormal behaviour.

Conditions. 16:8 h L:D (665 lux), 30-minute transition period. Aeration. Feeding with live brine shrimp nauplii three times a day and two times a day from day 26.

Chemical analysis. Water samples were taken at days 4, 7, 10, 11, 14, 21, 28 and 32. Radioactivity was determined by LSC followed by HPLC (LOQ 50 µg/L). Mean procedural recovery 79 %.

Calculations and statistics. Negative and sovent control groups were compared using the Student's t-test or 2 x 2 contingency tables. Discrete variables of treatment groups were analysed using 2 x 2 contingency tables. Continuous variable data were evaluated for normality using chi-square test or Kolmogorov-Smirnov test. Homogeneity of variance was calculated using Bartlett's test.

Results

Mean measured concentrations 3.0, 6.5, 12, 28 and 54 µg equivalents/L. Test substance was stable under the conditions of administration (79 - 93 % of nominal, means of all measurements taken during the experiment). Measured concentratons were consistently rather low at day 0 (61 – 65 %). Variability was thought to be due to the difficulty of obtaining representative water samples. The test substance was apparently binding to any particulate/organic matter in the test aquaria in spite of all precautions taken to reduce the amount of particulate matter. Hatching in the negative and solvent controls 88 and 83%. In the test treatments the hatching success varied from 80 to 86%. No significant differences with the controls. Survival was between 76 and 95 % at 3.0 to 28 µg/L At 54 µg/L a survival was 22% and significantly lower than in the controls (79 and 86% for the negative control and the solvent control, respectively). Total length, wet weight and dry weight were significantly different from pooled control group at 28 µg/L. Significance was not determined at the highest concentration because of the significant effects on survivial. Nevertheless

effects had increased at the highest concentration. NOEC for length, wet and dry weight 12 µg/L, based on mean measured concentrations.

Remarks

Water quality criteria within accepted limits. The result NOEC 12 µg equivalents/L, based on mean measured concentrations.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.4 Chronic toxicity to aquatic invertebrates

4.4.4.1 Study 1 - Daphnia, chronic toxicity

Study reference: STUDY IIA 8.3.2/001

Detailed study summary and results

Type of study	:	Daphnia, chronic toxicity	GLP statement	:	yes
Year of execution	:	1994	Guideline	:	US EPA 540/9-82-024; ASTM E 1193-87; US EPA 540/9-86-141
Test substance	:	Technical MK-244 (emamectin benzoate), batch L-656,748-052 S-002, purity 97.5 %, appearance white powder Radiolabelled MAB1a ([^{3}H]MK-244), batch L-653,825-055J001, 15670-101-28/93-325	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg/L]
emamectin benzoate and radiolabelled MAB1a	*Daphnia magna*	flow-through	19.7-21.0	8.1 – 8.4	21	NOEC	0.088

Description

Methods. Chronic toxicity of emamectin benzoate to *Daphnia magna* was tested under flow-through conditions. Nominal concentrations 0.050, 0.10, 0.20, 0.40, 0.80 µg/L, control, solvent control (methanol 1 mL/L). Dilution water was well water with total hardness 128 - 132 mg $CaCO_3$/L, 325 - 330 µmhos/cm, 300 mL glass beakers, 6.5 volumes/d. Four replicates with 5 daphnids each and eight replicates containing one daphnid each. Three-weekly determinations of survival, reproduction and clinical signs of toxicity.

Conditions.16:8 h L:D with 30 min transition period (325 - 840 lux). Feeding with a mixture of yeast and trout chow as well as a suspension of green algae three times daily, continuous aeration.

Chemical analysis. Sampling at day 0, 7, 14 and 21 to determine radioactivity by LSC followed by HPLC. Recovery 85 – 104 %, LOQ in LSC 0.00169 – 0.00184 µg/L. LOQ in HPLC 0.2 mg as/L. Mean procedural recovery 109%.

Calculations and statistics. NOEC was determined by ANOVA and Student's t-test.

Results

Mean measured concentrations during the test ranged from 80 to 88% of nominal values. Mean measured concentrations corrected for mean procedural recovery 109%, mortality and total offspring are summarised in Table 4.4.4.1-1.

Table 4.4.4.1-1. Analytical and biological results

Nominal [µg/L]	Actual [µg/L]	Mortality [%]	Reproduction [total # juveniles/female]
control		0	107
solvent control		4	144
0.050	0.043	0	144
0.10	0.088	0	120
0.20	0.16	86[1]	102[2]
0.40	0.34	100[1]	-
0.80	0.67	100[1]	-

[1] Statistically significant at $P \leq 0.05$

[2] not included in comparisons due to reduction in survival

The reproduction results of the solvent control differed significantly from the negative control. Therefore the solvent control was used for comparisons among the treatment groups. Reproduction at 0.043 and 0.088 µg/L did not significantly differ from the solvent control.

NOEC was 0.088 µg/L based on daphnid survival and expressed on the basis of mean measured concentrations.

Remarks

Water quality parameters within accepted range. Concentrations not properly chosen, mortality at three highest concentrations. The result NOEC for survival of 0.088 µg/L, based on mean measured concentrations.

4.4.5 Chronic toxicity to algae or aquatic plants

Please refer to the acute toxicity studies with algae and aquatic plants.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Study reference: STUDY IIA 8.5.2/01

Detailed study summary and results

Type of study	:	toxicity sediment dwelling organisms	GLP statement	:	yes
Year of execution	:	2005 - 2006	Guideline	:	OECD 218; proposal BBA guideline (1995)

Test substance	:	MK-244 (emamectin benzoate), batch nr. EZ910012, purity 95.6 %, appearance -white powder	Acceptability	:	acceptable

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg as/kg dwt sediment]
emamectin benzoate B_{1a} and B_{1b}	*Chironomus riparius*	water/sediment spiked	19.8 – 20.5	7.4 – 8.6	29	$NOEC_{emergence}$ $NOEC_{development}$	1.25 10

Description

Methods. Chronic effects of emamectin benzoate (90.3% emamectin benzoate B_{1a} and 5.7% emamectin benzoate B_{1b}) on chironomid larvae were assessed in a water-sediment system under static conditions. Test systems contained 50 g dry weight sediment (70 g wet sediment with moisture content 39.2%) artificial sediment (20 % kaolin clay, 75.5 % industrial sand and 4.5 % sphagnum peat) and 250 ml M7 test medium, pH 6.9. Before spiking, the vessels were shaken to create a water-sediment slurry. Water-sediment slurry was spiked 25 µL of application solution giving nominal concentrations of 0.63, 1.25, 2.5, 5.0, 10 µg a.s./L, control and solvent control (acetone 0.1 mL/L). After spiking, the test vessels were transferred to a rolling mill for two hours and were then left to stand undisturbed for three days to allow the sediment to settle. The sediment layer was approximately 1.5 cm and a water column of approximately 4 cm depth. Aeration of the vessels 24 h before introduction of the larvae. Four replicate systems (500 mL glass jars) with 20 first instar larvae each. Incubations for 29 days, assessment for mortality, and emergence daily at day 10 to day 28., sex of emerged individuals determined.

Conditions. Temperature 20 °C, 16:8 h L:D (672 - 1066 lux) with 30 minute transition period, daily feeding with commercial fish feed, 0.5-1 mg/larvae, constant aeration.

Chemical analysis. Overlying water and sediment samples on days 0 (introduction of larvae) and 29, analysis by reversed-phase HPLC with mass spectroscopy detection, LOQ 0.05 µg/L in water, 0.5 µg/kg in sediment.

Calculations and statistics. Emergence ratios of the control and solvent control were compared with two-tailed Fisher's Exact test. NOEC was obtained with a two-tailed Fisher's Exact test. Probit analysis was used for determination of EC_{50}-value. Confidence limits for the EC_{50} estimate were determined using Fieller's method.

Results

Actual concentrations in the water were below the LOQ. In the sediment, measured concentrations of emamectin benzoate B1a were between 94 and 116% of nominal at day 0 and at day 29. Concentrations of emamectin benzoate B1b were below LOQ for all concentrations except for the highest concentration of 10 µg/L which was 100% of nominal. At test end the concentrations of emamectin B1a were between 83 and 101% of nominal. Concentrations of emamectin benzoate B1b were below LOQ for all concentrations. Mean emergence in controls was 84% and 85% in the solvent control. In the test substances the emergence (males + females) was 83%, 79%, 38%, 8% and 1% at increasing concentrations. Males and females pooled for statistical analyses. Also the two controls were pooled as these did not differ significantly. Emergence rate was significantly reduced at 2.5 µg/kg and higher. Development rate was not influenced at the tested concentrations. EC_{50} reported as 2.4 µg a.s./kg dwt sediment (95% C.I. 1.2 – 2.9) for emergence rate and a NOEC of 1.25 µg a.s/kg dwt sediment. For the development rate an EC_{50} of > 10 µg a.s./kg dwt sediment was determined for males and for males and females pooled. No EC_{50} was determined for females alone. The NOEC for males, for females and for males and females pooled was 10 µg as/kg dwt sediment.

Remarks

Concentrations in water phase below LOQ indicate high sorption of test compound to the sediment. The result NOEC 1.25 µg a.s/kg dwt sediment for emergence rate and 10 µg a.s/kg dwt sediment for development rate, based on nominal initial concentrations of emamectin benzoate B_{1a} in the sediment phase.