Rats (HsdBrlHan:WIST) (2/sex/dose) were exposed nose-only to abamectin at actual concentrations of 0, 1.03. 3.71, 9.59 and 24.7 µg/L for 6 h/day on 5 consecutive days. The MMAD of the particles ranged from 1.54 to 3.49 µm.

Clinical signs, body weights and food consumption were recorded daily. At the end of the 5-day period the animals were killed and macroscopically examined. Brains, liver, kidneys and lungs were weighed, and these organs and larynx, nasopharyngeal cavity, trachea and any other abnormal tissue were examined microscopically.

Results

Results from a 5-day inhalation study with abamectin in rats

Concentration (µg/L)	0		1.03		3.71		9.59		24.7		dr
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality								A	A	A	dr
Clinical signs during exposure[B] - tremors and tail erection (day 2) - increased breathing depth (day 2)									1/2	1/2	
Clinical signs after exposure[B,C]				1/2	1/2	1/2	2/2	2/2	2/2	2/2	dr
Body weight loss[B]								2/2	2/2	2/2	dr
Reduced food consumption[B]							2/2	2/2	2/2	2/2	dr
Organ weights	No toxicologically relevant effects										
Pathology											
- macroscopy	No toxicologically relevant effects										
- microscopy	No toxicologically relevant effects										

dr = dose related; Number of animals affected/number of animals tested.

A At 9.59 µg/L one female showed numerous clinical signs at day 3 (see B). This animal was killed on day 3 and necropsied. At 24.7 µg/L animals displayed severe clinical signs after exposure (see B). In this group the two females were killed on day 2 (one before exposure, one after 4.5h of exposure), and one male was killed on day 3 (before exposure). The surviving male was allowed to recover from day 3-6 (without further exposure).

B Number of animals affected/number of animals tested.

C A dose-dependent increase in number and severity of clinical signs was observed. Reduced splay reflex was observed in one female at 1.03 µg/L, and in one male and one female at 3.71 µg/L towards the end of the 5 days exposure period. At 9.59 µg/L one female displayed hunched posture and extremely reduced foot splay reflex after the second exposure, and numerous clinical signs at day 3. At this dose, the other female and the two males showed some clinical signs (e.g. [extremely] reduced foot splay reflex, tremors, decreased activity, hunched posture, piloerection) from days 2-6. At 24.7 µg/l all animals displayed clinical signs of toxicity. The

following signs were observed: shaking, (extremely) reduced stability, hunched posture, piloerection, decreased activity, pale skin, reduced breathing rate, tail erection, (extremely) reduced splay reflex, decreased visual placing response.

Acceptability

Acceptable as a preliminary study.

Conclusions

Daily inhalation exposure for 5 consecutive days induced dose-dependent increases in clinical signs after exposure at all doses. The severity of the clinical signs was such that at 9.59 and 24.7 µg/L (part of) the animals were humanely killed during the treatment period. At 9.59 and 24.7 µg/L a dose-dependent body weight loss and reduction in food consumption was observed.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA 6.3.3/ 02 section No.	Repeat dose inhalation	Official use only

Section A6.3 / 6.4 / 6.5 Annex Point IIA6.3 / 6.4 / 6.5	Repeat dose inhalation Section 6.3.3/02 Repeat Dose Inhalation, 30 day inhalation study in the rat	
Title:	30 Day Inhalation Toxicity Study In The Rat	
Lab Report Number:	No. MR0237	
Authors:	[redacted] (2006)	
Test Substance:	Abamectin technical	
Species:	Rat	
Guidelines:	US-EPA OPPTS 870.3465	
Date of Report:	August 2006	
Published:	No	
GLP:	Yes	

STUDY 2

Characteristics

Reference/notifier	:	[redacted] 2006b	Exposure	:	inhalation (nose only), 6h/day, 5 days/week, over a 30-day period
Type of study	:	short-term inhalation	Doses	:	actual concentrations: 0, 0.111, 0.577 and 2.69 µg/L
Year of execution	:	2006	Vehicle	:	none
Test substance	:	Abamectin technical, purity [redacted]	GLP statement	:	Yes
Route	:	inhalation	Guideline	:	US-EPA OPPTS 870.3465
Species	:	Rat (HsdBrlHan:WIST)	Acceptability	:	acceptable
Group size	:	10/sex/dose	NOAEL	:	0.577 µg/L (0.11 mg/kg bw/day)

Study design

Rats (HsdBrlHan:WIST) (10/sex/dose) were exposed nose only to abamectin at actual concentrations of 0, 0.111, 0.577 and 2.69 µg/L for 6 h/day, 5 days/week over a 30 day period (total of 21 exposures). The MMAD of the particles ranged from 1.73 to 2.43 µm. Clinical signs were recorded daily. Detailed clinical examination was performed weekly. Body weights and food consumption were recorded weekly. Ophthalmoscopy was performed prior to treatment and during week 4. The animals were subjected to a functional observational battery followed by a motor activity test during week 4. The day after the last exposure the animals were killed and macroscopically examined, and blood was collected for haematology and clinical chemistry. A selection of organs was weighed. An extensive range of organs and tissues of the control and high dose animals, and any abnormal tissue of the low- and mid-dose animals was microscopically examined.

Note: Treatment of the animals started at different days during the week. As a consequence, before the day of termination the animals had been exposed for 1-4 consecutive days, following a two-day treatment free period.

Results

Results from a 30-day inhalation study with abamectin in rats.

Concentration (µg/L)	0		0.111		0.577		2.69		dr
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	No toxicologically relevant effects								
Clinical signs								4/10[A]	
Body weight (gain)	No toxicologically relevant effects [B]								
Food consumption	No toxicologically relevant effects								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology	No toxicologically relevant effects								
Clinical Chemistry	No toxicologically relevant effects [C]								
Organ weights	No toxicologically relevant effects [D]								
FOB	No toxicologically relevant effects								
motor activity								-19%*	
Pathology									
- macroscopy	No toxicologically relevant effects								
- microscopy	No toxicologically relevant effects [E]								

dr = dose related. *: statistically significant

[A] Number of animals affected/number of animals tested. At 2.69 µg/L, one female was found prostrate, shaking and gasping, with a swollen head on day 2, before the start of the exposure. This animal was killed and necropsied. At 2.69 µg/L, one female was ungroomed, with stains around the mouth, hunched posture and piloerection on day 15 and two females had abnormal respiratory noise in week 5.

[B] A slight reduction in body weight (5%), observed in the high-dose females is considered not toxicologically relevant.

[C] A slight reduction (26%) in alanine aminotransferase (ALAT) in the high dose males is considered not toxicologically relevant.

[D] A slight increase in relative spleen weight (+8%) in high dose males is considered not toxicologically relevant. Occasionally slight increases in organ weights were observed in the 0.577 µg/L group. Since the effects were slight, not dose-dependent and not accompanied by histological changes, they are considered not toxicologically relevant.

[E] A slight increase in demyelination of the sciatic nerve in the 2.69 µg/L group was not statistically significant, and is considered not treatment-related.

Acceptability

The study is considered acceptable.

Conclusions

Based on the increased incidence in clinical signs and reduced motor activity in females of the 2.69 µg/L group, the NOAEL is 0.577 µg/L.

Based on a rat breathing rate of 45 L/kg bw/hour and an exposure of 5 days a week, the NOAEL is equivalent to 0.11 mg/kg bw/day ((0.577 x 45 x 6 : 1000) x 5/7) and the LOAEL to 0.52 mg/kg bw/day.

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*5 June 2008; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]

Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

4. SUBCHRONIC TOXICITY

98/8 Doc IIIA section No.	6.4.1/ 01	Subchronic oral toxicity	Official use only
91/414 Annex Point addressed	II 5.3.2 / 01	Subchronic oral toxicity	

Title:	Twelve-week oral range-finding study in dogs	
Lab Report Number:	TT 82-073-0	
Authors:	[redacted] (1984c):	
Test Substance:	Abamectin technical (MK-0936, [redacted]	
Species:	Dog	
Guidelines:	Not applicable (dose range-finding study)	
Date of Report:	3 July 1984	
Published:	No	
GLP:	No	

STUDY 1

Characteristics

Reference/notifier	:	[redacted] (1984c)	Exposure	:	12 weeks
Type of study	:	12 week oral range-finding study	Doses[1]	:	0, 0.25, 0.5, 1.0 and 4.0/2.0 mg/kg (0, 6, 13, 25, 100/50 ppm bw/day)
Year of execution	:	1982	Vehicle	:	acetone
Test substance	:	Abamectin technical (purity [redacted])	GLP statement	:	no
Route	:	Oral (diet)	Guideline	:	-
Species	:	Dog (beagle)	Acceptability	:	As range-finding study only
Group size	:	2/sex/dose	NOAEL	:	-

1: treatment of the 4.0 mg/kg bw/day dose group was discontinued for 9 days on day 20 and reinstated at a dose level of 2.0 mg/kg bw/day; only nominal concentrations were presented.

Study design

In this study, only body weight, food consumption, clinical signs and pupillary miotic response were determined. Only data on body weights were presented individually.
Because of decreased food consumption, weight loss and signs of toxicity at the high dose level, the animals in the 4.0 mg/kg bw/day dose group received untreated feed from days 20 through 28. From day 29 animals of the high dose group received diet containing 2.0 mg/kg bw/day abamectin.

Results

In animals dosed 4.0/2.0 mg/kg bw/day, food consumption was reduced by 75%/50%. During treatment with 4.0 mg/kg bw/day, in one female dog rapid respiration, disorientation, tremors, weakness and slight uncoordination was observed. These signs disappeared on suspension of dosing and did not reappear on commencement of treatment at 2.0 mg/kg bw/day. Animals in the high dose group lost weight or did not gain weight. On day 36, animals of the high dose group were sacrificed and discarded. Body weigh gain in the other groups were not affected; food consumption of these groups was not reported.
Absence of miotic response (absence of pupil constriction in response to direct light) was noted in animals dosed 1.0 and 4.0/2.0 mg/kg bw/day.

Acceptability

The study is acceptable as range-finding study only.

Conclusions

No clinical signs, effect on body weight gain or pupil response to light was observed in dogs dosed up to and including 13 ppm (0.5 mg/kg bw/day).

98/8 Doc IIIA 6.4.1/ 02 section No.	Subchronic oral toxicity	Official use only
91/414 Annex II Point addressed 5.3.2 / 02	Subchronic oral toxicity	

Title:	C-076(B1a) [=Avermectin B1a]: 18-week oral toxicity study in dogs C-076(B1a) 18-week oral toxicity study in dogs supplemental histology	
Lab Report Number:	TT 76-073-0 and TT 76-073-0 addendum	
Authors:	[redacted] (1976) [redacted] (1982) for addendum	
Test Substance:	C-076(B1a) [=Avermectin B1a]	
Species:	Dog	
Guidelines:	OECD guideline no. 409 (September 1998) and Council Directive 88/302/EEC, B.27, sub-chronic oral toxicity test in non-rodents. Deviations: Two valid dose groups only (dosing of 2 groups suspended after one and 3 doses). Treatment was for 18 weeks rather than 13 weeks. Groups of 3 animals/sex used. Food consumption not recorded	
Date of Report:	Original report dates not specified. Addendum dated 19 November 1982.	
Published:	No	
GLP:	No	

STUDY 2

Characteristics

Reference/notifier	:	[redacted] (1976); [redacted] (1982, supplemental histology)	Exposure	:	18 weeks
Type of study	:	18 week oral toxicity study in dogs	Doses[1]	:	0, 0.25, 0.5, 2.0 and 8.0 mg/kg bw/day
Year of execution	:	Not specified; supplemental histology: 1982	Vehicle	:	Sesame oil
Test substance	:	Avermectin B1a (purity not specified)	GLP statement	:	No (supplemental histology: yes)
Route	:	Oral (gavage)	Guideline	:	In accordance with OECD 409
Species	:	Dog (beagle)	Acceptability	:	acceptable
Group size	:	3/sex/dose	NOAEL	:	0.25 mg/kg bw/day

1: dose group 0.25 mg/kg bw/day was added on the second day of dosing; dose groups 2.0 and 8.0 mg/kg bw were suspended after three and one doses respectively; dosing of one 0.5 mg/kg bw/day male was suspended for 4 days in week 3; only nominal concentrations were presented.

Study design

The study is in accordance with OECD 409, with the following deviations: two valid dose groups only (dosing of two groups suspended after one and three doses), only 3 animals/sex/dose, food consumption was not recorded, age of the dogs was 26-42 weeks at the beginning of the study, no information is provided on the purity of the avermectin B_{1a} batches used, no information is provided on the light/dark regime and on the duration of the acclimation period, no histological examination of trachea, epedidymides, aorta and bone marrow, individual ECG data were not included.

Results

The results of the study are summarized in table below.

Results of 18-week oral toxicity study in dogs

Dose (mg/kg bw/day)	**0**		**0 sesame oil**		**0.25**		**0.50**		**2.0[a]**		**8.0[b]**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality								1	2	1	2	1	
Clinical signs													
- ataxia							+	+	+	+	+	+	
- whole body muscular tremors							+	+	+	+	+	+	
- mydriasis							+	+	+	+	+	+	
- ptyalism							+	+	+	+	+	+	
- tonic convulsion									+	+	+	+	
- emesis									+	+	+	+	
Body weight gain[c]							d		d				
Food consumption	Not recorded												
Water consumption	Not recorded												
Ophthalmoscopy	No toxicologically relevant effects												
Haematology[c]													
- haemoglobin											i		
- haematocrit											i		
- erythrocytes											i		
- leucocytes											i		

Clinical Chemistry[c] - glucose						i	
ECG[c, d] - QT interval - bradycardia						i +	
Urinalysis	No toxicologically relevant effects						
Organ weights	No toxicologically relevant effects						
Pathology							
- macroscopy	No toxicologically relevant effects						
- microscopy - diffuse vacuolisation hepatocytes - oedema of the gall bladder				1	1 1 1 1	2 1 2	

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly; + = present

a: dosing was stopped after the third dose

b: dosing was stopped after the first dose, samples taken 4 h post-dose on day 1

c: males and females combined

d: week 1

Acceptability

The study is considered acceptable.

Conclusions

Based on the observed mortality, clinical signs of toxicity, reduced weight gain and histopathological changes in the liver at and above 0.5 mg/kg bw/day, the NOAEL in this study with avermectin B1a is 0.25 mg/kg bw/day.

98/8 Doc IIIA 6.4.1/ 03 section No.	Subchronic oral toxicity	Official use only
91/414 Annex II Point addressed 5.3.2 / 03	Subchronic oral toxicity	

Title:	MK-936 Fifty-three week dietary toxicity study in dogs	
Lab Report Number:	TT 82-104-0	
Authors:	[redacted] (1984d)	
Test Substance:	Abamectin technical (MK-0936). batch no. [redacted]	
Species:	Dog	
Guidelines:	OECD guideline no. 452 (May 1981) and Council Directive 88/302/EEC, chronic toxicity test (May 1988). Deviations: The test method employed exceeds the requirements with the exception that plasma GGT activity was not determined.	
Date of Report:	15 June 1984	
Published:	No	
GLP:	Yes	

STUDY 3

Characteristics

Reference/notifier	:	[redacted] (1984d)	Exposure	:	52 weeks
Type of study	:	53 week oral toxicity study in dogs	Doses	:	0, 0.25, 0.5 and 1.0 mg/kg bw/day
Year of execution	:	1982/1983	Vehicle	:	acetone
Test substance	:	Abamectin technical (purity [redacted]	GLP statement	:	yes
Route	:	Oral (diet)	Guideline	:	In accordance with OECD 452
Species	:	Dog (beagle)	Acceptability	:	acceptable
Group size	:	6/sex/dose	NOAEL	:	0.25 mg/kg bw/day

Study design

The study is in accordance with OECD 452, with the following deviations: no data on abamectin concentration in the food; the feed of dogs treated at 1.0 mg/kg bw/day was supplemented with 200 g/day wet dog food from weeks 36 (f) and 39 (m); no information on water consumption

Results

The results of the study are summarized in table below.

Results of 53 week oral toxicity study in dogs

Dose (mg/kg bw/day	0		0.25		0.5		1.0		dr
Sex	m	f	m	f	m	f	m	f	
Mortality							3		
Clinical signs - pupil reactivity to direct light					d	d	d	d	
Body weight							d	d	
Food consumption							d	d	
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
Haematology	No toxicologically relevant effects								
Clinical Chemistry - urea - protein							d d	d d	
Urinalysis	No toxicologically relevant effects								
Organ weights	No toxicologically relevant effects								
Pathology									
- macroscopy	No toxicologically relevant effects								
- microscopy	No toxicologically relevant effects								

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly

Acceptability

The study is considered acceptable.

Conclusions

Based on the decreased/absence pupil reactivity to light at 0.5 mg/kg bw/day, the NOAEL in this study is, in accordance with the opinion of the notifier, 0.25 mg/kg bw/day.

Justification below not/partially reported in DAR

Section 6.4.1 **Subchronic oral toxicity** **Annex Point IIA 6.4.1/04**	
JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only

Section 6.4.1	**Subchronic oral toxicity**
Annex Point IIA 6.4.1/04	
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**
Limited exposure [ ]	**Other justification [ ]**
Detailed justification:	[redacted]

Section 6.4.2 Annex Point IIA 6.4.2/01	Subchronic dermal toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [ ]	
Detailed justification:	[redacted]	

Section 6.4 Annex Point IIA 6.4.2/02	Subchronic inhalation toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	Technically not feasible [ ] Scientifically unjustified [ ]	
Limited exposure [ ]	Other justification [ ]	

Section 6.4 Annex Point IIA 6.4.2/02	Subchronic inhalation toxicity
Detailed justification:	[redacted]

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*5 June 2008; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...

Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Summary (copied from the abamectin PPP DAR)

An 8-week dietary range-finding study in the rat and 12, 18 and 53-week toxicity studies in the dog have been performed by dietary, gavage and dietary administration respectively. The data from the first 12 weeks of the rat 2-year study are not considered, since in a long term toxicity study less parameters are studied compared to a short term/semichronic toxicity study, and are not intended to replace a short term/semichronic toxicity study, as suggested by the notifier. A 90-day toxicity study in rats was not conducted. The studies were performed using abamectin technical except the 18 week toxicity study in dogs which used avermectin B1a.

Table 6.3.4-1 Subacute toxicity studies

Test substance	Duration, route	Species	NOAEL (mg/kg bw/day	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Notifier
Abamectin technical (vehicle acetone)	8 weeks, oral	rat	-	-	Range-finding study (only bw, food consumption and clin. signs)	[redacted] (1984b)

Table 6.3.4-2 Semichronic toxicity studies

Test substance	Duration, route	Species	NOAEL (mg/kg bw/day	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Notifier
Abamectin technical (acetone)	12 weeks, oral	dog	-	-	Range-finding study (only bw, food consumption and pupil respons)	[redacted] (1984c)
Avermectin B1a (vehicle sesame oil)	18 weeks, oral	dog	0.25	0.5	Mortality, clinical signs of toxicity (ataxia, tremors, mydriasis, ptyalism), reduced weight gain, histopathological changes in the liver	[redacted] [redacted] (1976)
Abamectin technical (vehicle acetone)	53 weeks, oral	dog	0.25	0.5	Absent or decreased pupil reflex (death at 1.0 mg/kg bw/day)	[redacted] (1984d)

Only the 18 week and 53 week oral toxicity studies with dogs are considered relevant, since the 8 week and 12 week study were range finding study, with determination of very few parameters, not conform OECD guidelines.

In the 18 week oral toxicity study with dogs, a very steep dose-response relationship for avermectin B1a in the dog was observed, since the oral NOAEL by gavage is 0.25 mg/kg bw/day and death, clinical signs (ataxia, tremors, mydriasis and ptyalism), reduced weight gain and histopathological changes in the liver occurred at 0.5 mg/kg bw/day.
In the 53-week oral toxicity study with abamectin technical in dogs, death occurred at the high dose level of 1.0 mg/kg bw/day, and pupil reactivity was decreased or absent at the dose level of 0.5 mg/kg bw/day. Based on this effect on pupil reactivity, the NOAEL in this study is 0.25 mg/kg bw/day.The results of both these studies show that a similar steep dose response exists for abamectin technical.
Therefore, the most appropriate NOAEL in the short-term toxicity studies is 0.25 mg/kg bw/day for both abamectin technical and avermectin B1a in the dog.

Justification for no oral 90-day toxicity study in the rat

In contrast to the suggestion of the notifier, the data from the first 12 weeks of the rat 2-year study are not considered to replace a 90-day study, since in a long-term toxicity study less parameters are studied compared to a short term/semichronic toxicity study, and are not intended to replace a short term/semichronic toxicity study. However, it is not likely that a 90 day toxicity study will give additional information to the information of the other toxicity studies. Furthermore, the dog is more sensitive than the rat (taking into account the range-finding study in rat and the 2-year study in rat) and the most appropriate short-term NOAEL is derived from the dog studies. Therefore, a 90-day toxicity study in rat for abamectin is not necessary.

a) 28-day and 90-day percutaneous toxicity studies in rats

no studies submitted.
Acute dermal toxicity studies with rat and rabbit has shown that abamectin has a low order of toxicity. A dermal penetration study with monkeys has shown that less than 1% of abamectin is absorbed through the skin. Based on these findings, percutaneous exposure will not be a significant route of exposure. Therefore, the lack of 28-day and 90-day percutaneous toxicity studies in rats is considered not to constitute a data gap.

b) 28-day and 90-day inhalation toxicity studies in rats

A 5-day range-finding inhalation toxicity study and a 30-day inhalation toxicity study in rats were performed. The results of the 30-day inhalation study show that in the highest dose group clinical signs and reduced motor activity were observed. The NOAEL is 0.577 µg/L (equivalent to 0.11 mg/kg bw/day).

No 90-day inhalation toxicity study has been performed.
Although in acute inhalatory toxicity studies it has been shown that abamectin is very toxic by inhalation, exposure data show that inhalation of abamectin is not a significant route of exposure. Furthermore, a 30-day inhalation toxicity study in rats is available. Therefore, the lack of a 90-day inhalatory toxicity study in rats is considered not to constitute a data gap.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*5 June 2008; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

5. CHRONIC TOXICITY

98/8 Doc IIIA 6.5/ 01 section No.	**Chronic Toxicity + Carcinogenicity**	**Official use only**
91/414 Annex II Point addressed 5.5 / 01	Carcinogenicity and chronic toxicity	

See the study summaries on chronic toxicity and carcinogenicity in rats and mice, presented under '7. Carcinogenicity'.	

6. GENOTOXICITY

In DAR: STUDY 1 (B.6.4 Genotoxicity (Annex IIA 5.4), B.6.4.1 In vitro; this study summary is copied from the DAR for completeness, but is not present in the initial Doc IIIA from the notifier)

Study design and results

Type of study: bacterial reverse mutation assay

Indicator cells	Endpoint	Result -act	Result +act	Activation		Dose range[1]	Reference Notifier
				tissue	inducer		
TA 1535 TA 1537 TA 1538 TA 98 TA 100	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -	Rat liver	Aroclor 1254	3, 10, 30, 100, 300 and 1000 µg/plate solvent: DMSO	[redacted] (1986a)
Test substance: avermectin B1 (MK-0936, purity [redacted]) GLP: yes According to OECD 471: yes, deviations: the amount of cells/ml was not given, the viable cell numbers were not determined, individual plate counts were not included.							

1: the highest dose is based on the observation in a previous study (not submitted) of marked precipitation at higher concentrations. However, precipitation was also observed at the highest test dose in this study.

Acceptability

In spite of the deviations of OECD guideline 471 (the amount of cells/ml was not given, the viable cell numbers were not determined, individual plate counts were not included), the study is considered acceptable.

Conclusions

Avermectin B1 did not induce gene mutations in the strains of *S. Typhimurium* used in the study at doses up to and including 1000 µg/plate.

98/8 Doc IIIA 6.6.1/ 01 section No.	In vitro gene mutation study in bacteria	Official use only
91/414 Annex II Point addressed 5.4.1 / 03	In-vitro gene mutation study in bacteria – Bacterial reverse mutation assay	

Title:	MK 936 tech.- Salmonella and *Escherichia*/mammalian-microsome mutagenicity test	
Lab Report Number:	20002072	
Authors:	Deparade, E. (2001)	
Test Substance:	Abamectin technical, [redacted]; Purity: [redacted].	
Species:	Histidine-auxotrophic strains of *Salmonella typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537 and the tryptophan-auxotrophic strain WP2 uvrA of *Escherichia coli*	
Guidelines:	OECD 471 (1997), EPA OPPTS 870.5100 (1998), Council Directive 92/69/EEC, B.14 (1992), JMAFF (2000)	
Date of Report:	12 September 2001	
Published:	No	
GLP:	Yes	

STUDY 2

Study design and results

Type of study: bacterial reverse mutation assay

Indicator cells	Endpoint	Result -act	Result +act	Activation		Dose range[1]	Reference Notifier
B: *S. typh.*				Rat liver	Aroclor 1254	312.5, 625.0, 1250.0, 2500.0 and 5000.0 µg/plate solvent: DMSO	Deparade, E. (2001)
TA 98	point mut.	-	-				
TA 100	point mut.	-	-				
TA 102	point mut.	-	-				
TA 1535	point mut.	-	-				
TA 1537	point mut.	-	-				

B: *E.coli* WP2 uvrA	point mut	-	-				
Test substance: abamectin technical (MK 936 tech., purity [redacted]) GLP: yes According to OECD 471: yes, deviation: no statistical tests were performed on the study using *E. coli*							

1: the highest dose is based on the results of a preliminary toxicity test. Precipitation of the test substance was observed at concentrations of 1250 to 5000 µg/plate.

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, abamectin technical did not induce point mutations in *S. Typhimurium* and in *E. coli*.

In DAR: **STUDY 10** (B.6.8 Further toxicological studies (Annex IIA 5.8), B.6.8.1 Toxicity studies of metabolites. B.6.8.1.2 Reproductive toxicity; this study summary is copied from the DAR for completeness, but is not present in the initial Doc IIIA from the notifier)

Study design and results

Type of study: microbial mutagenesis assay

Indicator cells	Endpoint	Result -act	Result +act	Activation		Dose range[1]	Reference Notifier
B: *S. typh.* TA 98 TA 100 TA 1535 TA97a B: *E.coli* WP2 WP2 uvrA WP2 uvrA pKM101	 point mut. point mut. point mut. point mut. point mut point mut point mut	 - - - - - - -	 - - - - - - -	Rat liver	Aroclor 1254	0, 10, 30, 100, 300, 1000 and 3000 µg/plate solvent: DMSO	Gordon, L.R. (1988c)
Test substance: 8,9-Z isomer of avermectin B1a (purity [redacted]) GLP: yes According to OECD 471: yes, deviation: no justification provided for independent confirmatory test for negative result.							

1: the highest dose is based on the observations in a previous study of precipitation of the parent compound, abamectin, at concentrations >1000 µg/plate;

precipitation was observed at 3000 µg/plate

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, the 8,9-Z isomer of avermectin B1a did not induce point mutations in *S.*

Typhimurium and in *E. coli*.

98/8 Doc IIIA 6.6.2/ 01 section No.	**In vitro cytogenicity study in mammalian cells**	**Official use only**
91/414 Annex II Point addressed 5.4.1 / 04	*In-vitro* cytogenicity study in mammalian cells	

Title:	Avermectin B1 (MK-0936) Assay for chromosomal aberrations in vitro in Chinese hamster ovary cells	
Lab Report Number:	TT 85-8631 and TT 85-8632 (range-finding) and TT 85-8635 (main study)	
Authors:	Gordon, L. R. (1986b)	
Test Substance:	Abamectin technical ([redacted]	
Species:	Chinese hamster ovary cells (clone WBL)	
Guidelines:	OECD guideline no. 473 (July 1997) and Council Directive 2000/32/EEC, B.10, *in vitro* mammalian chromosome aberration test Deviations: exposure time in the second assay remained at 3 hours, rather than continuous treatment until harvest single cultures were assayed for each concentration, but no historical data are presented in the report to demonstrate minimal variation between cultures	
Date of Report:	11 March 1986	
Published:	No	
GLP:	Yes	

STUDY 3
Study design and results

Type of study: mammalian chromosome aberration test

Indicator cells	Endpoint	Result -act	Result +act	Activation		Dose range[a]	Reference Notifier
				tissue	inducer		
Chinese hamster ovary cells (CHO-WBL)	Chromosome aberrations	-	-	Rat liver	β-naphta-flavone and phenobarbital	-S9: 0.0100, 0.0150, 0.0200, 0.0250, 0.0300 and 0.0350 mM +S9: 0.0050, 0.0100, 0.0150, 0.0200 and 0.0250 mM Solvent: DMSO	Gordon, L.R. (1986b)
-S9: >65% reduction in monolayer confluence and large decreases in number of mitotic cells were observed at and above 0.0250 mM, and because of toxicity, cells of these dose groups were not scored for aberrations. +S9: at 0.0200 mM there was less than 20% reduction in monolayer confluence with no obvious decrease in number of mitotic cells, whereas at 0.0250 mM, >90% reduction in monolayer confluence and obvious suppresion of mitotic cells was observed (cells of this group were not scored for aberration). Test substance: avermectin B1 (MK-0936, purity [redacted] composition B1a: [redacted] and B1b: [redacted]), exposure 3h. GLP: yes According to OECD 473: yes, deviations: 200 cells per concentration were determined only in S9-activated cells harvested at 10.5 h, whereas 100 cells per concentration were determined in non-S9-activated cells harvested at 10.5h and 24h.							

a: based on two range-finding cytotoxicity assays.

Acceptability

In spite that only 100 cells per concentration were scored in the absence of S9, the study is considered acceptable.

Conclusions

Abamectin tecnical did not induce chromosomal aberration in mammalian cells.

SYNGENTA CONCLUSION

Conclusion:	Abamectin technical and/or its metabolites do not induce chromosome aberrations *in vitro* in CHO cells at concentrations up to 0.02mM.	

98/8 Doc IIIA 6.6.3/ 01 section No.	Genetic Toxicity – In Vitro	Official use only
91/414 Annex II Point addressed 5.4.1 / 01	In-vitro gene mutation assay in mammalian cells	

Title:	MK-936 V-79 mammalian cell mutagenesis	
Lab Report Number:	No. TT 82-8506, 82-8510, 82-8512 and 82-8519	
Authors:	Gordon, L. R.	
Test Substance:	Abamectin (batch no. [redacted], purity of batch not reported but specified elsewhere [redacted] 1984g] as [redacted] by HPLC)	
Species:	Not applicable	
Guidelines:	Test method conforms to OECD guideline no. 476 (July 1997) and 2000/32/EEC, B.17, in vitro mammalian cell gene mutation test, with the following exceptions: Only 2 of 4 concentrations tested with S9 in the repeat experiment had >10% cell survival Due to a formulation error and 1 of 4 concentrations showing high cytotoxicity, only one valid concentration tested without S9 in the repeat experiment	
Date of Report:	15 March 1983	
Published:	No	
GLP:	Yes	

STUDY 4
Study design and results

Type of study: mammalian cells *in vitro*, gene mutations, HGPRT-assay

Indicator cells	Endpoint	Result -act	Result +act	Activation		Dose range[a]	Reference Notifier
				tissue	inducer		
Chinese hamster lung cells (V79)	Gene mutation (HGPRT)	-	-	Rat liver	Aroclor 1254	-S9[1]: 0.003, 0.004, 0.005 and 0.006 mM +S9: 0.03, 0.04, 0.045 and 0.05 mM Solvent: DMSO	Gordon, L.R. (1983)

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

Test substance: abamectin (MK-0936, purity [redacted]), exposure 3h.
GLP: yes
According to OECD 476: yes, deviations: only 2 of 4 concentrations tested with S9 in the repeat experiment had >10% cell survival; due to a dilution error and 1 of 4 concentrations showing high cytotoxicity, only one valid concentration was tested without S9 in the repeat experiment.

a: based on cytotoxicity range-finding studies.

Acceptability

In spite of the deviations of the OECD guideline 476, the study is considered acceptable.

Conclusions

Abamectin did not induce gene mutations in mammalian cells *in vitro*.

SYNGENTA CONCLUSION

Conclusion:	Abamectin and/or its metabolites are not mutagenic at the V-79 cell HGPRT locus, based on the absence of a reproducible ≥3-fold increase in relative mutation frequency and no evidence of a dose-response relationship at concentrations up to those eliciting marked cytotoxicity.	

Reliability Indicator	1	
Data Protection Claim	Yes	

Evaluation by Competent Authorities

	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*8 november 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
	[redacted]
[redacted]	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Justification below not/partially reported in DAR

98/8 Doc IIIA section No.	6.6.4	If positive in 6.6.1, 6.6.2 or 6.6.3, then an in-vivo mutagenicity study will be required (bone marrow assay for chromosomal damage or a micronucleus test)
91/414 Annex Point addressed	II 5.4.2/01	Genetic Toxicity – Additional *in vivo* mutagenicity studies

Section 6.6.4 **Annex Point IIA 6.6.4**		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8 November 2007	
Evaluation of applicant's justification	-	
Conclusion	[redacted]	

Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

98/8 Doc IIIA section No.	6.6.5/ 01	Genetic Toxicity – In Vivo	Official use only
91/414 Annex Point addressed	II 5.4.2 / 01	Genetic Toxicity – *in vivo* mammalian bone marrow chromosome aberration test	

Title:	An assessment of the mutagenic potential of MK-0936 utilizing the *in vivo* mice bone marrow-cytogenetics assay	
Lab Report Number:	No. LSC-5608; TT 83-900-6.	
Authors:	Blazak, W. F	
Test Substance:	Abamectin (MK-0936, batch no. [redacted], purity [redacted])	
Species:	Mice	
Guidelines:	Test method conforms to OECD guideline no. 475 (July 1997) and 2000/32/EEC, B.11, *in vivo* mammalian bone marrow chromosome aberration test.	
Date of Report:	13 June 1983	
Published:	No	
GLP:	Yes	

STUDY 1

Study design and results

Type of study: chromosome aberration test

Species	Endpoint	Result	Dose range[1]	Reference Notifier
Mouse (male CD-1)	Structural chromosome aberration	-[2]	0, 1.2, 4.0 and 12 .0 mg/kg bw	Blazak, W.F. (1983)
Test substance: abamectin technical (MK-936, purity [redacted]) Vehicle: sesame oil GLP statement: yes According to OECD 475: yes, with the following deviations: colchicine was given 2 h prior to sacrifice (should be 3-5h); only male mice were studied whereas at the time of the study there are no data available from studies with mice that demonstrate that there is no substantial difference in toxicity between sexes; a maximum of 50 cells/animal were evaluated for chromosomal aberrations (should be at least 100 cells/animal); the weight variation of the mice exceeded the range given in the study protocol.				

1: based on the results of a pilot study.

2: the observed significant increase in cells with aberration and frequency of chromosomal aberrations per cell in the 1.2 mg/kg bw group is considered incidental, since the increases were only observed at 6h and no increases were observed at higher doses.

Persistent tremors were observed from 6h until 24h after treatment in animals given 12 mg/kg bw. At the 24h sacrifice, mean bodyweight was significantly lower in mice from the 4.0 and 12.0 mg/kg bw groups.

Acceptability

In spite of the deviations from OECD guideline 475 (colchicine was given 2 h prior to sacrifice, only male mice were studied whereas at the time of the study there are no data available from studies with mice that demonstrate that there is no substantial difference in toxicity between sexes; a maximum of only 50 cells/animal were evaluated for chromosomal aberrations) the study is considered acceptable.

Conclusions

Under the study conditions, abamectin technical does not induce cytogenic damage in male mouse bone marrow cells.

SYNGENTA CONCLUSION

Conclusion:	Abamectin and/or its metabolites do not induce cytogenetic damage in male mouse bone marrow cells even at acute oral dose levels up to the maximum tolerated dose.	

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*8 November 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]

Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Justification below not/partially reported in DAR

98/8 Doc IIIA section No.	6.6.6/01	If positive in 6.6.4 then a test to assess possible germ cell effects may be required
91/414 Annex Point addressed	IIA 5.4.3	*In vivo* studies in germ cells

Section 6.6.6 **Annex Point IIA 6.6.6**		
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	[redacted]	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	8 November 2007	
Evaluation of applicant's justification	-	
Conclusion	[redacted]	

Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Summary of genotoxicity (copied from the abamectin PPP DAR)

The genotoxicity studies which are considered acceptable for the overall evaluation of abamectin are summarized in Tables below.

In vitro genotoxicity studies

Indicator cells	Endpoint	Result without activation	Result with activation	Reference Notifier
S. typhimurium (5 strains)	Point mutation	-	-	Gordon, L.R. (1986f)
S. typhimurium (5 strains) & *E.coli (1 strain)*	Point mutation		-	Deparade, E. (2001)
Chinese hamster ovary cells	Chromosome aberration	-	-	Gordon, L.R. (1983g)
Chinese hamster V-79 cells	Gene mutation (HGPRT)	-	-	Gordon, L.R. (1983a)

- : result is negative

In vivo genotoxicity studies

Species	Endpoint	Result	Reference Notifier
CD-1 strain mouse bone marrow	Chromosome aberrations (*in vivo*)	Negative	[redacted] (1983)

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

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*7 november 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

7. CARCINOGENICITY

98/8 Doc IIIA 6.7/ 01 section No.		Chronic Toxicity + Carcinogenicity	Official use only
91/414 Annex Point addressed	II 5.5 / 01	Carcinogenicity and chronic toxicity	

Title:	MK-936 105-week dietary carcinogenicity and toxicity study in rats with a 53-week interim necropsy	
Lab Report Number:	No. TT 82-099-0	
Authors:	[redacted]	
Test Substance:	Abamectin (batch no. [redacted], initial purity [redacted] by LC, purity determined in week 51, [redacted])	
Species:	Rats	
Guidelines:	Test method conforms to OECD guideline no. 453 (May 1981) and 88/302/EEC, B.33, combined chronic toxicity/carcinogenicity test, with the following exceptions: 15 animals/sex/group used for sacrifice after 52 weeks Mortality was 42 - 68% at termination after 104 weeks GGT activity not measured in interim kill animals.	
Date of Report:	27 August 1985	
Published:	No	
GLP:	Yes	

STUDY 1
Characteristics

Reference/notifier	:	[redacted] 1985b)	Exposure	:	104 weeks
Type of study	:	Carcinogenicity and toxicity study in rats.	Doses	:	0, 0, 0.75, 1.5 and 2.0[3] mg/kg bw/day
Year of execution	:	1982-1984	Vehicle	:	acetone
Test substance	:	Abamectin technical (purity [redacted])	GLP statement	:	yes

Route	:	Oral (diet)	Guideline	:	OECD 453
Species	:	Rats (Sprague-Dawley derived Crl:CD(SD)BR strain)	Acceptability	:	acceptable
Group size	:	50/sex/dose and 15/sex/dose for interim necropsy	NOAEL	:	1.5 mg/kg bw/day

a: the high dosage level was increased to 2.5 mg/kg bw/day in week 11, but due to the appearance of severe signs of CNS toxicity, the dosage level was decreased back to 2.0 mg/kg bw/day in week 13 for the remainder of the study.

Study design

The dose levels employed were selected on the basis of the results of a range-finding study ([redacted], 1984b; Point 6.3.1). The highest dose level was increased to 2.5 mg/kg bw/day for weeks 11 and 12, followed by one day off-dose. Fifteen animals/sex/group were designated for interim sacrifice at week 53, and 50 animals/sex/group for the oncogenicity study sacrificed after 104 weeks treatment. Mortality checks and clinical observations were made daily and detailed physical examinations, including palpation, weekly. Body weights were recorded pre-test and weekly thereafter. Food consumption was measured weekly in 12 animals/sex/group for 5 or 6 days/week. The eyes of all animals were examined pre-test and subsequently, control and high dose group animals eyes were examined in weeks 26, 52/53, 76 and 102/103. Haematological, serum chemistry and urinalysis investigations were performed at weeks 12, 25, 38 and 51 in 10 animals/sex/group designated for sacrifice after 52 weeks, in week 78 on 10 animals/sex/group from the main study animals, and pre-terminally in all survivors. All decedent and surviving animals were subjected to detailed necropsy and *post mortem* examination. Organ weights were recorded for all animals that survived to scheduled sacrifice. Tissue/organ samples from all animals sacrificed at 52 weeks, those that died or were killed during the study and all animals killed at the end of the study were preserved.
Microscopic examination of tissues was performed on all control (I) and high dose animals killed at 52 weeks, all decedents from all groups scheduled to be killed after 52 weeks, and all decedents and survivors from all groups scheduled to be killed after 104 weeks. Mortality and body weight data (104-week groups only) were statistically analysed by the Mantel-Haenszel trend test, and tumour incidences by the extended Mantel-Haenszel procedure implementing adjustments for variables.

Results

The results of the study are summarized in table below.

Results of carcinogenicity and toxicity study in rats.

Dose (mg/kg bw/day)	0 (I)		0 (II)		0.75		1.5		2.0		dr
Sex	m	f	m	f	m	f	m	f	m	f	
Mortality (%)	52	62	52	48	62	68	42	66	68	64	
Clinical signs - whole body tremors - unthrifty appearance									 i i	 i i	
Body weight gain (%)[1]					21	30	9	14	6	13	
Food consumption	No toxicologically relevant effects										

Water consumption	Not performed	
Ophthalmoscopy	No toxicologically relevant effects	
Haematology	No toxicologically relevant effects	
Clinical Chemistry	No toxicologically relevant effects	
Urinalysis	No toxicologically relevant effects	
Organ weights	No toxicologically relevant effects	
Pathology		
- macroscopy	No toxicologically relevant effects	
- microscopy	No toxicologically relevant effects	

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly

1: data of males: at termination, data of females: at week 60

Mortality was higher than 50% in all but two groups of animals. Compared to control groups, mortality incidence was higher in males of the 2.0 mg/kg bw/day group. Whole body tremor and unthrifty appearance were confined to the highest dose group and, in all but one instance, first occured in week 12 after the dose level was increased to 2.5 mg/kg bw/day. The clinical signs persisted intermittently until termination.

A treatment-related increase in weight gain was observed in both sexes of all treated groups, the effect being inversely related to the dose. Higher weight gains occured in male groups throughout the treatment period and at termination the overall weight gains were significantly increased. In females, the increased weight gain was confined to the first 60 weeks of the treatment, and at termination the overall weight gains of female groups were not significantly different from controls. The increases in body-weight gain are considered treatment-related, but is not considered as an adverse effect.

No neuromuscular changes were found to account for the clinical signs.

There was no significant increase in tumor incidence resulting from treatment with abamectin.

Acceptability

In spite of the deviations from OECD guideline 453, there are no individual data on clinical signs and at the end of the study, mortality was higher than 50% in all but two groups, the study was considered acceptable.

Conclusions

The NOAEL in this study is 1.5 mg/kg bw/day, based on the increased incidence in mortality in males and observed clinical signs in the highest dose group. Abamectin is not carcinogenic in this study.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA 6.7/ 02 section No.	Chronic Toxicity + Carcinogenicity	Official use only
91/414 Annex II Point addressed 5.5 / 02	Carcinogenicity and chronic toxicity	

Title:	MK-936 94-week dietary carcinogenicity and toxicity study in mice	
Lab Report Number:	No. TT 83-002-0/-1/-2/-3	
Authors:	[redacted]	
Test Substance:	Abamectin (MK-0936, batch no. [redacted], initial purity [redacted] by HPLC, purity determined in week 34 [redacted] w/w)	
Species:	Mice	
Guidelines:	Test method conforms to OECD guideline no. 451 (May 1981) and 88/302/EEC, B.32, carcinogenicity test.	
Date of Report:	27 August 1985	
Published:	No	
GLP:	Yes	

STUDY 2
Characteristics

Reference/notifier	:	[redacted] (1985c)	Exposure	:	89/93 weeks (males), 93 weeks (females
Type of study	:	Carcinogenicity and toxicity study in mice.	Doses[1]	:	0, 0, 2.0, 4.0 and 8.0 mg/kg bw/day
Year of execution	:	1983-1984	Vehicle	:	acetone
Test substance	:	Abamectin (purity [redacted]	GLP statement	:	yes
Route	:	Oral (diet)	Guideline	:	OECD 451
Species	:	Mouse (Crl:CD-1 (ICR)BR)	Acceptability	:	acceptable
Group size[2]	:	74/sex/dose	NOAEL	:	4.0 mg/kg bw/day

1: dose levels were selected on the basis of results from a 12-week range-finding study in mice (not submitted)

2: 12 animals/sex/group were sacrificed at 6 and 12 months to obtain blood samples for haematology and clinical chemistry examinations.

Study design

Groups of 74 male and 74 female 6-week-old mice (Crl:CD-1 (ICR)BR strain) were administered abamectin (MK-0936, batch no. [redacted]) orally for 93 weeks, by admixture in the diet, at concentrations adjusted to provide dose levels of 0 (control I), 0 (control II), 2.0, 4.0 and 8.0 mg/kg bw/day. All female groups started on study were killed and discarded shortly after the initiation of treatment due to the presence of tremors at all dose levels within 24 hours, and deaths at 4.0 and 8.0 mg/kg bw/day. New groups of females were started on test, at the same dose levels, approximately one month later. Treatment of the male group with 8.0 mg/kg bw/day was discontinued after 89 weeks when survival had reached 40%. The dose levels employed were selected on the basis of results from a 12-week range-finding study in mice (TT 82-082-0/-1/-2; not submitted).

Twelve animals/sex/group were killed for blood sampling in week 25/26, and a further 12 animals/sex/group in week 52. Mortality checks and clinical observations were made daily and detailed physical examinations, including palpation, weekly. Body weights were recorded pre-test and weekly thereafter. Food consumption was measured weekly in 12 animals/sex/group for 6 days/week. The eyes of all animals were examined pre-test and subsequently, control and high dose group animal eyes were examined in weeks 51/53 and 91. Haematological and serum chemistry investigations were performed in weeks 25/26 and 52 in 12 animals/sex/group, in all moribund animals after week 69, and pre-termination for all surviving animals. All animals killed after 26 and 52 weeks for blood sampling were subjected to necropsy, gross post mortem examination and tissue preservation only. All decedent and surviving animals scheduled for sacrifice after 93 weeks were subjected to detailed necropsy and post mortem examination. Organ weights were recorded for all animals that survived to scheduled sacrifice. Tissue/organ samples were preserved for all animals that died or were killed during the study and all animals killed at the end of the study were preserved. Microscopic examination of tissues, gross lesions and palpated masses was performed on all decedents and survivors from all groups scheduled to be killed after 93 weeks.

Mortality and body weight data (93-week groups only) were statistically analysed by the Mantel-Haenszel trend test. Tumour incidences were analysed by the extended Mantel-Haenszel procedure implementing adjustments for variables.

Results

The results of the study are summarized in table below.

Results of carcinogenicity and toxicity study in mice.

Dose (mg/kg bw/day)	0 (I)		0 (II)		2.0		4.0		8.0		dr
Sex	m	f	m	f	m	f	m	f	m	f	
Mortality (%)	50	28	50	32	48	40	48	40	68 is	32	

Clinical signs - tremors					i	
Body weight gain (%)					-7 ds	-21 ds	
Food consumption			i	i	i	dr
Water consumption	Not performed					
Ophthalmoscopy	No toxicologically relevant effects					
Haematology - Ht					i	
Clinical Chemistry - glucose[1]					d	d	
Urinalysis	Not performed					
Organ weights (rel) - spleen - adrenals - thyroid - kidneys - pituitary - ovaries			d i i	d i i	i i d	i i i	
Pathology - macroscopy	No toxicologically relevant effects					
- microscopy - dermatitis incidence (%) - spleen: extramedullary hematopoiesis	4 5/50	6 4/49	6 8/49	12 8/50	22 15/50	dr

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly

1: determined at week 93, no data from weeks 25/26 and 52.

Among the males, there was a higher incidence of mortality in the 8 mg/kg bw/day group compared to the controls, and treatment of this group was discontinued after 89 weeks. The two most common causes of death or sacrifice were lymphoma and amyloidosis. There were no specific treatment-related pathologic changes in the death animals that could account for the increased mortality in this group.

Tremors occured in several females from the original treatment group after one day. On day two, 3 and 7 females died in dose groups 4 and 8 mg/kg bw/day, respectively. Treatment was withdrawn, and females were replaced. Treatment-related tremors recurred in some females of all groups. All female mice were terminated and new groups of females restarted on study 4 weeks later. Following restart of the study, sporadic tremors were observed in 2 females of the highest dose group, at the end of the study period.

An overall reduced body weight gain of 7% in males and of 21% in females was observed in animals of the highest dose group, compared with the mean weight gain of the control groups. In females only, food consumption was increased by 2% in the lowest dose group to 8% in the highest dose group. In the highest dose group, plasma glucose values were lower compared to controls, and in high dose males an increase in haematocrit value was observed.

Changes in organ weights were observed for spleen, ovaries, adrenals, thyroid, kidneys and pituitary, but were not dose-related, and no histopathological changes were observed in these organs. The observed higher spleen weight in males of the highest dose group is likely to be related to the observed increased

haematopoiesis in the spleen in males of this group.
In males of dose groups 4 and 8 mg/kg bw/day, a dose-related increase in dermatitis was observed. There was no increase in tumor incidences.

Acceptability

The study was considered acceptable.

Conclusions

Based on the observed effects in the highest dose group, increase in mortality in males, extramedullary haematopoiesis in the spleen in males and decrease in body weight gain in males and females, the NOAEL in this study is 4.0 mg/kg bw/day. Abamectin is not carcinogenic in this study.

Reliability Indicator	1	
Data Protection Claim	Yes	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*8 november 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
	[redacted]
	[redacted]
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*

Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

8. REPRODUCTIVE TOXICITY

98/8 Doc IIIA section No.	6.8.1/ 01	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex Point addressed	II 5.6 2 / 01	Oral teratogenicity	

Title:	MK-936 Oral range-finding study in pregnant rats and oral teratogenic study in rats	
Lab Report Number:	No. TT 82-705-1 and TT 82-705-0	
Authors:	[redacted]	
Test Substance:	Abamectin ([redacted], purity approximately [redacted] by UV spectrophotometry)	
Species:	Rat	
Guidelines:	The method employed conforms to OECD draft guideline 414 (August 1999) and 88/302/EEC, B.31, with the following exceptions: Daily recording of clinical observations performed during dosing period only No statistical analysis of incidences of malformations and variations No gross observations at necropsy of maternal animals Also deviates from OECD draft guideline 414 in respect of: Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Visceral examination by dissection performed on approximately one-third of foetuses No historical control data reported to substantiate specific conclusions	

Date of Report:	10 November 1982	
Published:	No	
GLP:	Yes	

STUDY 1

Characteristics

Reference/notifier	:	[redacted] (1982a)	Exposure	:	Day 6 to 17 of gestation[a] (range-finding study) Day 6 to 19 of gestation[a] (teratogenicity study)
Type of study	:	Oral range-finding study in pregnant rats and oral teratogenicity study in rats with abamectin technical.	Doses	:	0, 0.25, 0.5, 1.0 and 2.0 mg/kg bw (range-finding study) 0, 0.4, 0.8 and 1.6 mg/kg bw/day (teratogenicity study)
Year of execution	:	1982	Vehicle	:	Sesame oil
Test substances	:	abamectin technical (MK-936, purity approx. [redacted])	GLP statement	:	Yes
Route	:	Oral (gavage)	Guideline	:	OECD 414
Species	:	Rats (CRCD)	Acceptability	:	Acceptable
Group size	:	10f/dose (range-finding study) 25f/dose (teratogenicity study)	$NOAEL_{mat}$ $NOAEL_{dev}$	: :	1.6 mg/kg bw/ day 0.8 mg/kg bw/ day

a: day 0 of gestation = ady plug or sperm in vaginal smear observed.

Study design

The study was performed in accordance with OECD guideline 414, with the following deviations: clinical observations during dosing period only, no statistical analysis of incidences of malformations and variations, no gross observations at necropsy of maternal animals, non-gravid uteri not stained for occult implantation sites, uterus/cervix not weighed, visceral examination by dissection performed on approximately one-third of the fetuses, no historical control data reported to substantiate specific conclusions.

In the range-finding study, the animals were observed for clinical signs during the treatment period. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. The animals were killed on day 20 and their pregnancy status established.

In the teratogenic study, animals were observed for clinical signs during the treatment period. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 20 of gestation The animals were killed on day 20 and their pregnancy status established. The number of corpora lutea, the presence of resorptions and dead foetuses were examined. Fetuses were weighed, sexed and examined for external abnormalities, and visceral and skeletal abnormalities and variations.

Results

Range-finding study: one female of the highest dose group was killed in a moribund condition on day 18 of gestation. Prior to sacrifice, the animal had lost weight, apeared weak and displayed tremors. There was a treatment-related increase in maternal weight gain during the treatment period at all, except the highest, dose levels. This is considered not to be an adverse effect. Pregnancy incidences were not affected by abamectin.

The results of the teratogenicity study are summarized in table below.

Results of an oral teratogenicity study in pregnant rats with abamectin technical.

Dose (mg/kg bw/day)		0	0.4	0.8	1.6	dr
Maternal effects	Mortality	No mortality				
	Clinical signs	No toxicologically relevant effects				
	Pregnant animals	No toxicologically relevant effects				
	Abortions	none				
	Gravid uterine weight	Not performed				
	Corpus lutea	No data				
	Body weight gain (day 6-14)		is	is	is	
	Food consumption	Not performed				
	Water consumption	Not performed				
	Pathology	Not performed				
Litter response	Live fetuses	No toxicologically relevant effects				
	Fetal weight (g) (litter mean)	3.88	3.88	3.73	3.89	
	Pre implantation loss	No toxicologically relevant effects				
	Fetal implantation loss	No toxicologically relevant effects				
	Total no. resorptions	11	10	37	13	
	Resorptions/implants	0.03	0.03	0.06	0.03	
Fetus examination	No. of dead fetuses/no. of fetuses studied	0/319	0/320	0/279	0/326	
	Sex ratio (m:f)	1 : 0.94	1 : 0.95	1 : 0.98	1 : 0.83	
	Malformations -exencephaly -cleft palate			 1[b]	 1[a] 1	
	Skeletal deviations -lumbar rib -lumbar count variation	 44 1	 41 1	 45 1	 72 5	

a: conjoined twin

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

Teratogenicity study: maternal weight gain was increased from day 6-14 of gestation in all treated groups, but this is considered not an adverse effect. The sex ratio (m:f) was lower in the highest dose group. Since exposure to abamectin was from days 6-19 of gestation, abamectin could not have affected the sex of the fetuses directly. Apparently, abamectin exposure in the highest dose group affected resorption in a sex-specific way (more effect on female fetuses), resulting in a lower m:f ratio.

In the 0.8 mg/kg bw/day group a significant higher incidence of resorptions and decreased fetal weight were observed. A similar effect not was observed at 1.6 mg/kg bw/day, and therefore these effects are considered incidental. In the highest dose goup of this study, exencephaly is observed paired with a conjoined twin, and

thus it is possible that this effect is not substance-related*. The observed incidence of cleft palate in the highest dose groups is considered treatment-related, since this effect is also observed in other studies with abamectin and/or the main isomer of abamectin. In mice studies (see section B6.8) an increase in cleft palate is observed at and above 0.1 mg/kg bw/day. Furthermore, historical control data provided by the notifier in 2005 showed that in 23 studies only one fetus with cleft palate was observed.
In the highest dose group, the number of pups with lumbar rib and with lumbar count variation had increased.

*In 2005 the notifier submitted additional information and historical control values. He did, however, not submit historical control data on exencephaly in rats.

Acceptability

The study is considered acceptable.

Conclusions

Based on the absence of effects in the highest dose group, the NOAEL for maternal toxicity in this study is 1.6 mg/kg bw/day.
Based on the occurrence of cleft palate, changed sex ratio and increased number of fetuses with lumbar rib and lumbar count variation in the highest dose group, the NOAEL for developmental toxicity in this study is 0.8 mg/kg bw/day.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA section No.	6.8.1/ 02	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex Point addressed	II 5.6 2 / 02	Oral teratogenicity	

Title:	MK-936 Oral range-finding study in pregnant rabbits and oral teratogenic study in rabbits	
Lab Report Number:	No. TT 82-706-1 and TT 82-706-0	
Authors:	[redacted]	
Test Substance:	Abamectin (MK-0936, batch no. [redacted] purity approximately [redacted] by UV spectrophotometry)	
Species:	Rabbit	
Guidelines:	The method employed conforms to OECD draft guideline 414 (August 1999) and 88/302/EEC, B.31, with the following exceptions: No statistical analysis of incidences of malformations and variations No gross observations at necropsy of maternal animals. Also deviates from OECD draft guideline 414 in respect of: Numbers of pregnant animals <20/group Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed No historical control data reported to substantiate specific conclusions	
Date of Report:	10 November 1982	
Published:	No	

GLP:	Yes	

STUDY 2

Characteristics

Reference/notifier	:	[redacted] (1982b)	Exposure	:	Day 6 to 18 of gestation[a] (range-finding study) Day 6 to 27 of gestation[a] (teratogenicity study)
Type of study	:	Oral range-finding study in pregnant rabbits and oral teratogenicity study in rabbits with abamectin technical.	Doses	:	0, 0.5, 1.0, 2.0 and 3.0 mg/kg bw (range-finding study) 0, 0.5, 1.0 and 2.0 mg/kg bw/day (teratogenicity study)
Year of execution	:	1982	Vehicle	:	sesame oil
Test substances	:	abamectin technical (MK-0936, purity approx[redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 414
Species	:	Rabbits (New Zealand albino)	Acceptability	:	aceptable
Group size	:	10f/dose (range-finding study) 18f/dose (teratogenicity study)	NOAEL $_{mat}$ NOAEL $_{dev}$	: :	1.0 mg/kg bw/ day 1.0 mg/kg bw/ day

a: day 0 of gestation = day of insemination

Study design

The study was performed in accordance with OECD guideline 414, with the folllowing deviations: no statistical analysis of incidences of malformations and variations, no gross observations at necropsy of maternal animals, non-gravid uteri not stained for occult implantation sites, uterus/cervix not weighed, no historical control data reported to substantiate specific conclusions.

In the range-finding study, the animals were observed for clinical signs throughout the study. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18, 19 and 28 of gestation. The animals were killed on day 28 and their pregnancy status established.

In the teratogenicity study, animals were observed for clinical signs throughout the study. Body weights were recorded on days 0, 6, 9, 12, 15, 18, 21, 24, 27 and 28 of gestation. The animals were killed on day 28 and their pregnancy status established. The number of corpora lutea, the presence of resorptions and dead foetuses were examined. Fetuses were weighed, sexed and examined for external abnormalities, and visceral and skeletal abnormalities and variations.

Results

Range-finding study: one female of the highest dose group was killed in a moribund condition on day 16 of gestation. Prior to sacrifice, the animal had lost weight and was prostrate with laboured respiration and discharges from the nose and mouth. The other animals of the highest dose group displayed stuporous behavior 2 to 5 h after the fourth and subsequent doses, and some animals showed discharge from the nose and mouth and reduced water and food consumption. Abortion was observed at and above 1.0 mg/kg

bw/day (see note). Statistical significant weight loss was observed in animals of the highest dose group during the treatment period. Pregnancy incidences were not affected by abamectin.
Note: the notifier considers this effect not substance-related, since spontaneous abortion has occured in historical control groups. However, there are no data available on historical controls.

The results of the teratogenicity study are summarized in table below.

Results of oral teratogenicity study in rabbits with abamectin technical.

Dose (mg/kg bw/day)		0	0.5	1.0	2.0	dr
Maternal effects	Mortality	0	1	1	1	
	Clinical signs	No toxicologically relevant effects				
	Pregnant animals/mated	16/18	16/18	13/18	17/18	
	Abortions	No toxicologically relevant effects				
	Gravid uterine weight	Not performed				
	Corpus lutea	No data				
	Body weight (day 6-18)				ds	
	Food consumption				d	
	Water consumption				d	
	Pathology	Not performed				
Litter response	Live fetuses	No toxicologically relevant effects				
	Fetal weight	No toxicologically relevant effects				
	Resorptions/implants (litter mean)	0.049	0.038	0.036	0.065	
	Pre implantation loss	No toxicologically relevant effects				
	Foetal implantation loss	No toxicologically relevant effects				
	Post implantation loss	No toxicologically relevant effects				
Fetus examination	No. of abnormal fetuses	No toxicologically relevant effects				
	No. of dead fetuses/no. of fetuses studied	0/97	1/91	5/100	0/121	
	Sex ratio (m:f)	1 : 0.98	1 : 1.07	1 : 1.17	1 : 1.02	
	% malformed fetuses	3.1	4.4	4.0	12.4	
	External observations and visceral deviations -cleft palate -clubbed fore-foot -omphaloceles	 0 1 1	 0 0 0	 0 2 0	 2 5 2	
	Skeletal deviations -sternebral malformation -incompletely ossified sternebra -incompletely ossified metacarpal -incompletely ossified phalanx	 0 17 8 19	 0 17 15 27	 0 16 7 12	 3 42 33 31	

Teratogenicity study: Two deaths and one premature sacrifice occurred in abamectin-treated groups. Death was preceded by reduced food and water consumption in 2 animals and by blood-stained urine in the cage of the other animal. The relationship of these deaths to treatment with abamectin is equivocal since a dose-related increase in incidence did not occur. There were no clinical signs of toxicity at any dose level.
The food and water consumption of all groups was variable, but by subjective assessment, the periods of reduced food and water consumption in the group treated at 2.0 mg/kg bw/day were more prolonged and

pronounced than in the other groups. This treatment-related maternotoxicity at 2.0 mg/kg bw/day manifested as decreased food and water consumption resulted in a substantial weight loss during the dosing period which was statistically significant between day 6 and 18 of gestation compared to control.
There were no treatment-related effects at any dose level on pre-implantation loss and post implantation loss, and mean foetal weight (sexes combined) at any dose level. Higher numbers of dead fetuses and an increased m/f sex ratio was observed in the group treated at 1.0 mg/kg bw/day, but not at the higher dose level. Therefore, these effects are considered incidental.
In the high dose group, the number of resorptions and the % malformed fetuses were increased. The external malformations in the high dose group comprised 2 fetuses with cleft palate and 2 fetuses with omphalocele all from a single litter and 5 fetuses with clubbed fore-feet from 3 other litters. The incidences of these malformations are higher than the concurrent and historical control groups (not available) and were considered treatment related (by the study author). In addition, one fetus with clubbed fore-feet had a lumbar vertebral malformation and 3 of the fetuses in the litter with cleft palate and omphalocele had sternebral malformations, including one of the fetuses with cleft palate.
Two fetuses in one litter from a female treated at 1.0 mg/kg bw/day also had clubbed fore-feet but the occurrence is considered not to be treatment-related because higher incidences of the defect have been recorded in historical controls (not available), one fetus from a concurrent control female also had a clubbed fore-foot, and no other malformations were observed at this dose.
At 2.0 mg/kg bw/day, increased incidences of incomplete ossification of sternebrae and metacarpals are considered to reflect a treatment-related slight delay in ossification.

Acceptability
The study is considered acceptable.

Conclusions
The NOAEL of abamectin technical for maternal toxicity in rabbits in this study is 1.0 mg/kg bw/day, based on decreased water and food consumption and weight loss during gestation in the high dose group.
The NOAEL for foetal toxicity was also established at 1.0 mg/kg bw/day based on the occurrence of increased number of resorptions, delayed ossification and excess incidences of cleft palate, omphalocele and clubbed fore-feet at the maternally toxic dose level of 2.0 mg/kg bw/day.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA 6.8.1/ 03 section No.	**Reproductive Toxicity – Tests on developmental toxicity**	**Official use only**
91/414 Annex II Point addressed 5.8.1/ 03	Oral teratogenicity	

Title:	8,9-isomer of avermectin B1 oral teratology study in mice	
Lab Report Number:	No. TT 85-710-0	
Authors:	[redacted]	
Test Substance:	8,9-Z isomer of Avermectin B_{1a} [redacted] purity [redacted] by HPLC)	
Species:	Mice	
Guidelines:	The method employed conforms to OECD draft guideline 414 (August 1999) and 88/302/EEC, B.31, with the following exceptions: Daily recording of clinical observations performed during dosing period only Pre-implantation loss not evaluated No statistical analysis of incidences of malformations and variations Also deviates from OECD draft guideline 414 in respect of: Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Visceral examination by dissection performed on approximately one-third of foetuses No statistical analysis of incidences of malformations and variations Treatment continued up to day 15 of gestation, caesarean sections performed on day 17.	
Date of Report:	8 January 1986	

Published:	No	
GLP:	Yes	

In DAR : **STUDY 3** (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1986f)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.	Doses	:	0, 0.015, 0.03 and 0.06 mg/kg bw
Year of execution	:	1985	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a [redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 414 (draft)
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	25 mated females/dose	NOAEL $_{maternal}$	:	0.06 mg/kg bw/ day
			NOAEL $_{fetal}$	:	0.015 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

Study design

Four groups of 25 naturally-mated female mice (Crl:CF1 (BR) strain), were treated orally, by gavage, with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0 (vehicle only), 0.015, 0.03 and 0.06 mg/kg bw/day from day 6 to day 15 of gestation. Clinical signs were recorded daily on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, implantations were counted and classified as resorptions, dead foetuses or live foetuses. All foetuses were examined externally, weighed and sexed. Visceral examination by dissection was performed on every third foetus of each litter and on all externally abnormal foetuses. The head of every third foetus was fixed for examination, and all foetuses were fixed and examined for skeletal malformations and variations. Maternal body weight data and litter parameters were analysed statistically by ANOVA or ANCOVA using a least significant difference procedure after normalisation of non-parametric data.

Results

The results of the study are summarized in tables below.

Results of oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.

Dose level (mg/kg bw/day):	0	0.015	0.03	0.06

No. pregnant / no. mated	No toxicologically relevant effects			
No. aborted	0	0	0	1
Total no. implantations	No toxicologically relevant effects			
Mean no. implantations/litter	No toxicologically relevant effects			
Total no. resorptions	49	32	25	48
Total no. dead foetuses	0	1	0	0
% resorbed+dead foetuses / implant (litter mean)	18.4	18.2	9.7	19.3
Total no. live foetuses	No toxicologically relevant effects			
No. live foetuses/litter	No toxicologically relevant effects			
Sex ratio (M:F)	1 : 0.94	1 : 0.84	1 : 1.10	1 : 0.98
Live fetal weight (g) (litter mean)	0.93	0.90	0.89	0.87

Results of oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.

Dose level (mg/kg bw/day):	**0**	**0.015**	**0.03**	**0.06**
External examination:				
No. foetuses (litters) examined	200 (22)	210 + 1* (22)	231 (23)	212 (22)
No. foetuses (litters) with malformations	1 (1)	5 (2)	4 (4)	3 (2)
- no. (%) with exencephaly	0 (0.00)	0 (0.00)	3 (1.30)	3 (1.42)
- no. (%) hindlimb extension	1 (0.50)	4 (1.90)	1 (0.43)	0 (0.00)
- no. (%) cleft palate	0 (0.00)	1 (0.48)	0 (0.00)	0 (0.00)
% malformed foetuses	1.50	4.27	3.46	2.36
No. foetuses with sites of incomplete ossification	6	6	6	8
Total no. incompletely ossified sites:	7	7	15	17
- sternebrae	1	4	6	5
- vertebrae	4	1	3	8

* dead foetus

There were no deaths or treatment-related clinical signs. One female of the highest dose is considered to have aborted between days 10 and 12 of gestation, which is considered incidental. Body weight gain and litter parameters were unaffected by treatment. Three fetuses in each of the groups treated at 0.03 and 0.06

mg/kg bw/day had exencephaly, in 3 and 2 litters, respectively. The incidences are higher than both the concurrent control group and the historical control group. Similar malformations as occurring in the treated groups are also observed in other studies (See B6.8.1.2 studies 1 and 2) and indicate treatment-related teratogenicity.
The % malformed fetuses was increased in the treated groups, however inversely dose-related.
The incidence of incompletely ossified sites was higher in the 0.03 and 0.06 mg/kg bw/day groups. Incomplete ossification of vertebrae was higher in the 0.06 mg/kg bw/day group, whereas in all treated groups incomplete ossification of sternebrae was observed.

Acceptability

The study is acceptable as investigative study.

Conclusions

Based on the absence of maternal toxicity at the dose levels tested, the NOAEL for maternal toxicity in this study is 0.06 mg/kg bw/day.
Based on the observed higher incidences of exencephaly and increased number of incomplete ossified sites in fetuses of the two highest dose groups, the NOAEL for fetal toxicity in this study is 0.015 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	A no-observed-effect-level for both maternal and foetal toxicity, including teratogenicity, was established in the CF-1 Mice as >0.06 mg/kg bw/day, based on the absence of maternal and foetal toxicity at the highest dose level employed.	

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA 6.8.1/ 04 Reproductive Toxicity – Tests on developmental toxicity section No.	**Official use only**

91/414 Annex Point addressed	II 5.8.1/ 04	Oral teratogenicity	

Title:	8,9-isomer of avermectin B1 oral teratology study in mice	
Lab Report Number:	TT 85-710-1	
Authors:	[redacted]	
Test Substance:	8,9-Z isomer of Avermectin B_{1a} ([redacted] purity [redacted] by HPLC)	
Species:	Mice	
Guidelines:	The method employed conforms to OECD draft guideline 414 (August 1999) and 88/302/EEC, B.31, with the following exceptions: Daily recording of clinical observations performed during dosing period only Pre-implantation loss not evaluated Incomplete statistical analysis of incidences of malformations and variations Also deviates from OECD draft guideline 414 in respect of: Treatment continued up to day 15 of gestation, caesarean sections performed on day 17 Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Visceral examination by dissection performed on approximately one-third of foetuses.	
Date of Report:	8 January 1986	
Published:	No	
GLP:	Yes	

In DAR : STUDY 4 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity) **(text in purple: changes made after the PRAPeR expert meeting for PPP)**

Characteristics

Reference/notifier	:	[redacted] (1986g)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.	Doses	:	0, 0.015, 0.03, 0.10 and 0.50 mg/kg bw/day
Year of execution	:	1985	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a ([redacted])	GLP statement	:	yes
Route	:	Oral (gastric intubation)	Guideline	:	OECD 414 (draft)
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	25 mated females/dose	NOAEL maternal	:	~~0.015~~ 0.1 mg/kg bw/ day
			NOAEL fetal	:	~~0.015~~ 0.03 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

Study design

According to OECD guideline 414 (draft), with the following deviations: daily recording of clinical observations performed during dosing period only, pre-implantation loss not evaluated, incomplete statistical analysis of incidences of malformations and variations, treatment continued up to day 15 of gestation with caesarean sections performed on day 17, non-gravid uteri not stained for occult implantation sites, uterus/cervix not weighed and visceral examination by dissection performed on approximately one-third of fetuses.

Five groups of 25 naturally-mated female mice were treated orally, by gavage, with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0 (vehicle only), 0.015, 0.03, 0.10 and 0.50 mg/kg bw/day from day 6 to day 15 of gestation. Clinical signs were recorded on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation and food consumption was measured periodically. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, implantations were counted and classified as resorptions, dead foetuses or live foetuses. All foetuses were examined externally, weighed and sexed. Visceral examination by dissection was performed on every third foetus of each litter and on all externally abnormal foetuses. The head of every third fetus was fixed for subsequent examination. All foetuses were examined for skeletal malformations and variations.

Results

The results of the study are summarized in tables below.

Results of oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.

Dose level (mg/kg bw/day):	**0**	**0.015**	**0.03**	**0.10**	**0.50**
No. pregnant / no. mated	No toxicologically relevant effects				
No. aborted	none				
No. prematurely killed pregnant	0	0	0	0	1
Total no. implantations	No toxicologically relevant effects				
Mean no. implantations/litter	No toxicologically relevant effects				
Total no. resorptions	27	22	40	27	38
Total no. dead foetuses	No toxicologically relevant effects				
% resorbed+dead foetuses/implant (litter mean)	9.0	8.3	16.3*	9.3	14.3
Total no. live foetuses	261	283	238	279	233
No. live foetuses/litter	11.3	11.8	10.3	11.6	10.1
Sex ratio (M:F)	1 : 0.79	1 : 0.97	1 : 1.02	1 : 0.75	1 : 0.73
Live foetal weight (g) (litter mean)	No toxicologically relevant effects				

* $p < 0.05$

Results of oral teratology study in mice with the 8,9-Z isomer of avermectin B1a.

Dose level (mg/kg bw/day):	**0**	**0.015**	**0.03**	**0.10**	**0.50**
External examination:					
No. foetuses (litters) examined	261 (23)	283 + 2* (24)	238 + 1* (23)	279 (24)	233 + 1* (23)
No. foetuses (litters) with malformations	1 (1)	2 + 1* (3)	6 (3)	7 (2)	27 + 1* (9)
- no. with exencephaly	1	1 + 1*	5	0	1 + 1*
- no. with open eyelid	1	1	3	1	0
- no. with celft palate	0	1	1	6**	24***
- no. with cleft lip	0	0	0	1	0
- no. with micrognathia	0	0	0	0	1
- no. with tail malformation	0	0	0	0	1
No. foetuses (litters) with variations	none				
Visceral examination:					
No. foetuses (litters) examined	78 (23)	90 + 2* (24)	78 + 1* (23)	90 (24)	88 + 1* (23)
Malformations	No toxicologically relevant effects:				
No. fetuses (litters) with malformations	1 (1)	1 (1)	0	0	1 (1)

- no. with interrupted aortic arch					1
- no. with agenesis of testis		1			
- no. with hepatocellular necrosis	1				
No. foetuses (litters) with variations	none				
Skeletal examination:					
No. foetuses (litters) examined	261 (23)	283 + 2* (24)	238 + 1* (23)	279 (24)	233 + 1* (23)
Malformations	No toxicologically relevant effects:				
No. fetuses (litters) with malformations	4 (4)	7 + 1* (5)	4 (2)	3 (2)	1 (1)
- no. with cervical vertebra malform.	0	0 + 1*	0	0	0
- no. with thoracic vertebra malform.	0	0 + 1*	0	1****	0
- no. with missing vertebra	0	3	0	1****	0
- no. with fused rib	0	0 + 1*	0	1****	0
- no. with agenesis of rib	0	0 + 1*	0	1****	0
- no. with hypoplastic rib	0	0	0	1****	0
- no. with misshapen rib	0	0 + 1*	0	0	0
- no. with sternebral malformation	4	4	4	2	1
No. foetuses (litters) with variations	No toxicologically relevant effects				
No. foetuses with sites of incomplete Ossification	1	1	11	7	2
Total no. incompletely ossified sites:	2	1	16	13	2
- vertebrae	0	0	1	5	0
- skull bone	1	1	10	2	1
- sternebrae	1	0	5	6	1
TOTAL NO. MALFORMED FETUSES (external, visceral, skeletal)	**6**	**10 + 1***	**9**	**9**	**28 + 1***
% malformed fetuses	**2.30**	**3.86**	**3.78**	**3.23**	**12.4**

* dead fetus

*** 6 fetuses with cleft palate from 1 litter

***** 24 fetuses with cleft palate from 6 litters

******* malformations observed in 1 fetus

One animal treated at 0.5 mg/kg bw/day was killed in a moribund condition after receiving 6 doses. The animal had marked weight loss, anorexia, lethargy and chromodacryorrhea prior to death. No other deaths or treatment-related clinical signs occurred during the study. Maternal body weight and food consumption

was unaffected by treatment at all dose levels and there were no treatment-related gross changes at necropsy. Embryonic survival and foetal weights were not significantly different from the controls. Compared with the control value of resorbed or dead fetuses/implantation (9%), an increase was observed in the groups treated at 0.03 and 0.50 mg/kg bw/day (16.3% and 14.3%, respectively).
An increased incidence of cleft palate occurred in the groups treated at 0.1 and 0.5 mg/kg bw/day (6 and 24, respectively) compared to the control group incidence of 0, indicating treatment-related teratogenicity. Exencephaly occurred at higher incidences in the group treated at 0.03 mg/kg bw/day. The incidences of visceral and skeletal malformations did not indicate an effect of treatment at any dose level. At the two highest dose levels, 0.1 and 0.5 mg/kg bw/day, all but one malformation (2 fetuses with sternebral malformation at 0.1 mg/kg bw/day) occurred in single fetuses. Higher numbers of fetuses with sites of incomplete ossification at 0.03 and 0.1 mg/kg bw/day are considered incidental to treatment because the incidence at the highest dose level, 0.5 mg/kg bw/day, was similar to the control incidence.

Acceptability

The study is acceptable as investigative study.

Conclusions

~~The NOAEL for maternal toxicity for the 8,9-Z isomer of avermectin B1a in CF-1 mice in this study was established as 0.015 mg/kg bw/day, based on the occurrence of increased number of resorptions at 0.03 mg/kg bw/day and 0.50 mg/kg bw/day. The NOAEL for teratogenic effects in this study is 0.015 mg/kg bw, based on increased incidence of malformations at and above 0.03 mg/kg bw (exencephaly at 0.03 and cleft palate at 0.1 and 0.5 mg/kg bw/day).~~

PRAPeR 39 (Dec. 2007): Considering that the number of foetuses with exencephaly was not dose related, only increased at 0.03 and not at higher dose levels, the experts agreed to set the foetal NOAEL at 0.03 mg/kg bw/day based on increased incidence of cleft palates (this is in agreement with the value adopted by JMPR in 1997).
Considering that the increase in resorptions observed at 0.03 mg/kg bw/day was not dose-related and most likely not related to maternal toxicity, the experts agreed to set the maternal NOAEL at 0.1 mg/kg bw/day based on the mortality observed at the high dose level (this is in agreement with the value adopted by JMPR in 1997).

SYNGENTA CONCLUSION

Conclusion:	A no-observed-effect-level (NOEL) for maternal toxicity in CF-1 mice was established as 0.1 mg/kg bw/day, based on the occurrence of one treatment-related death at 0.5 mg/kg bw/day. A frank teratogenic effect, characterised by an increased incidence of cleft palate, occurred in response to treatment with the 8,9-isomer of avermectin B_{1a} at 0.5 mg/kg bw/day. The minimum teratogenic dose level is 0.1 mg/kg bw/day based on a slight increase in the incidence of cleft palate. Therefore, a clear NOEL for teratogenicity in CF-1 mice was established as 0.03 mg/kg bw/day.	

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA section No.	6.8.1/ 05	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex Point addressed	II 5.8.1 / 07	Oral teratogenicity	

Title:	Delta-8,9-isomer of avermectin B1 - Oral developmental toxicity study in rats	
Lab Report Number:	No. TT 87-715-0	
Authors:	[redacted]	
Test Substance:	Abamectin ([redacted], purity [redacted] by HPLC)	
Species:	Rat	
Guidelines:	The method employed conforms to OECD draft guideline 414 (August 1999) and 88/302/EEC, B.31, with the following exceptions: Daily recording of clinical observations performed during dosing period only No gross observations at necropsy of maternal animals No statistical analysis of incidences of malformations and variations Also deviates from OECD draft guideline 414 in respect of: Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Visceral examination by dissection performed on approximately one-third of foetuses No historical control data reported to substantiate specific conclusions	

Date of Report:	1 June 1988	
Published:	No	
GLP:	Yes	

In DAR : STUDY 8 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1988a)	Exposure	:	Day 6-17 of gestation[1]
Type of study	:	Oral developmental toxicity study in rats.	Doses	:	0, 0.25, 0.5 and 1.0 mg/kg bw/day
Year of execution	:	1987	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a [redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 414 (draft)
Species	:	Rats (Crl:CD(SD) BR strain)	Acceptability	:	Acceptable as investigative study
Group size	:	25 mated females/dose	NOAEL $_{maternal}$	:	1.0 mg/kg bw/ day
			NOAEL $_{developm}$	:	1.0 mg/kg bw/ day

1: day 0 = day plug or sperm in vaginal smear observed

Study design

According to OECD guideline 414 (draft), with the following deviations: daily recording of clinical observations performed during dosing period only, no gross observations at necropsy of matarnal animals, no statistical analysis of incidences of malformations and variations, non-gravid uteri not stained for occult implantation sites, uterus/cervix not weighed, visceral examination by dissection performed on approximately one-third of foetuses.

Four groups of 25 naturally mated female rats (Crl:CD(SD) BR strain) were treated orally, by gavage, with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0 (vehicle only), 0.25, 0.5 and 1.0 mg/kg bw/day from day 6 to day 17 of gestation. The animals were observed for clinical signs daily from day 6 to day 20 of gestation. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. The food consumption of all animals was measured for 3-day intervals from day 3 to day 20 of gestation. The animals were killed on day 20 and subjected to a gross post mortem examination. Maternal gross lesions were preserved and subsequently examined microscopically. The pregnancy status was established and the number of corpora lutea determined. The uterine horns were examined and the number of implantation sites enumerated and classified as live foetus, dead foetus or resorption. The foetuses were removed from the uterine horns, individually weighed, sexed and examined for external malformations. Visceral examination by dissection was performed on every third foetus of each litter and on all externally abnormal foetuses.

The head of every third foetus was fixed for subsequent examination and all foetuses were examined for skeletal malformations and variations. Litter data and maternal body weight data were analysed statistically by ANOVA or ANCOVA.

Results

Results of the study are summarized in table below.

Results of oral developmental toxicity study in rats.

Dose (mg/kg bw/day)	**0**	**0.25**	**0.5**	**1.0**
Weight change days: 6 – 20	+113	+116	+122*	+120
No. pregnant / no. mated	25 / 25	24 / 25	25 / 25	25 / 25
% pre-implantation loss[a]	7.9	13.4*	6.2	6.6
External examination	No toxicologically relevant effects			
Visceral examination	No toxicologically relevant effects			
Skeletal examination	No toxicologically relevant effects			

* $p < 0.05$

There were no deaths and no treatment-related clinical signs during the study. Females treated at 0.5 and 1.0 mg/kg bw/day showed a treatment-related enhanced body weight gain during the treatment period. Food consumption was unaffected by treatment at all dose levels. There were no treatment-related gross changes at necropsy in maternal animals and pregnancy incidences were comparable between all groups. There were no treatment-related effects at any dose level on pre-implantation and post-implantation losses, live litter size, sex ratio and foetal weights. Pre-implantation loss in the group treated at 0.25 mg/kg bw/day (13.4%) was significantly higher than the control value of 7.9%, but because there was no dose-relationship and since implantation is essentially complete at the commencement of dosing, it is considered to be incidental to treatment. There was no evidence of developmental toxicity, either embryonic/foetal growth retardation or teratogenicity at any dose level based on the incidences of external, visceral and skeletal malformations, variations and unossified centres.

Conclusion

Based on the absence of adverse effects at the highest dose level tested, the NOAEL for maternal toxicicity and the NOAEL for developmental toxicity are both 1.0 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	A no-observed-adverse-effect-level (NOAEL) for maternal effects was established as >1.0 mg/kg bw/day, based on the absence of adverse effects at the highest dose level employed, 1.0 mg/kg bw/day. An NOEL for embryotoxicity including teratogenicity was established as >1.0 mg/kg bw/day, based on the absence of treatment-related malformations and foetal toxicity at the highest dose level employed.	

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA section No.	6.8.1/ 06	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex Point addressed	II 5.8.1 / 04	Oral teratogenicity	

Title:	8,9-isomer of avermectin B1 oral maternotoxicity study in mice	
Lab Report Number:	TT 84-722-1	
Authors:	[redacted]	
Test Substance:	avermectin B_{1a} ([redacted], purity [redacted] by HPLC)	
Species:	Mice	
Guidelines:	Not applicable (investigative study)	
Date of Report:	8 January 1986	
Published:	No	
GLP:	Yes	

In DAR : STUDY 2 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1986e)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral maternotoxicity study in mice with the 8,9-Zisomer of avermectin B1a.	Doses	:	0, 0.05, 0.1, 0.5 and 1.0 mg/kg bw/day
Year of execution	:	1984	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a [redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	unknown
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	12 mated females/dose	NOAEL $_{maternal}$	:	0.1 mg/kg bw/ day
			NOAEL $_{fetal}$	:	0.05 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

Study design

Five groups of 12 mated female mice (Crl:CF-1 (BR); 10 weeks old) were treated orally (gavage) with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0, 0.05, 0.1, 0.5 and 1.0 mg/kg bw/day from day 6 to day 15 of gestation. Clinical signs were recorded on working days. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, implantations were counted and classified as resorptions, dead fetuses or live fetuses. All fetuses were examined externally, weighed and sexed. Animals dying during the study were subjected to gross necropsy examination and determination of reproductive status only.

Results

The results of the study are summarized in tables below.

Results of oral maternotoxicity study in mice with the 8,9-Zisomer of avermectin B1a.

Dose (mg/kg bw/day):	**0**	**0.05**	**0.1**	**0.5**	**1.0**
Mean weight gain day 6 - 17		ds			ds
Overall pregnancy incidence	No toxicologically relevant effects				
Live pregnant	No toxicologically relevant effects				
No. (mean/female) implantations[a]	150 (12.5)	125 (10.4)	129 (11.7)	109 (12.1)	108 (9.8*)
No. resorptions	13	21	14	18	17
No. dead foetuses	No toxicologically relevant effects				
% resorptions + dead foetuses/implant	9.6	15.3	12.7	16.7	13.4
No (mean/female) live foetuses[a]	136 (11.3)	104 (8.7*)	116 (10.6)	90 (10.0)	91 (8.3*)
Sex ratio (m:f)	1 : 1.03	1 : 0.89	1 : 0.80	1 : 0.76	1 : 0.59
Mean live foetal weight (g)	No toxicologically relevant effects				

* $p < 0.05$

[a] includes females surviving to caesarean section

Results of oral maternotoxicity study in mice with the 8,9-Zisomer of avermectin B1a.

Dose (mg/kg bw/day)	**0**	**0.05**	**0.1**	**0.5**	**1.0**
No. foetuses (litters) examined	136 + 1* (12)	104 (12)	115 (11)	90 + 1* (9)	91 (11)
No. foetuses (litters) with malformations	2 (2)	1 (1)	15 (3)	5 (3)	10 (5)
No. foetuses (litters) with variations	none				
No. (%) with exencephaly	1 (0.7)	0	2 (1.7)	4 (4.4)	2 (2.2)
No. (%) with cleft palate	0	0	13 (11.3)	1 (1.1)	7 (7.7)

* dead foetus

Results: Two deaths occurred during the study, a female treated at 1.0 mg/kg bw/day was found dead on day 10 and one female treated at 0.5 mg/kg bw/day was killed on day 11 of gestation. Clinical signs prior to death were tremors (in one animal at 0.5 mg/kg bw), lethargy and weight loss. Other than in the 2 animals that died or were sacrificed prematurely, maternal body weight gain was unaffected by treatment at all dose levels. However, the mean weight gain from day 6 to day 17 of gestation in the groups treated at 0.05 and 1.0 mg/kg bw/day were significantly lower than the control group by 18.4%, due to significantly lower litter sizes. There were no treatment-related gross changes at necropsy in maternal animals of all treatment groups. The mean number of implantations/female in the group treated at 1.0 mg/kg bw/day (9.8) was significantly lower than the control value of 12.5. Fewer implantations/female also occurred in the group treated at 0.05 mg/kg bw/day. These differences from the control are considered not to be treatment-induced since implantation was essentially complete at the start of treatment on day 6 of gestation. The

incidence of resorptions was higher than the controls in all treated groups, but the differences were not statistically significant and their magnitude did not increase with dose. As a consequence of lower implantation numbers and higher incidences of resorption, the mean number of live foetuses/female was significantly reduced in the groups treated at 0.05 and 1.0 mg/kg bw/day. Since both contributing factors are considered not to be treatment-induced, the reduced live litter size is considered not to be related to treatment.

As also seen in the previous study (6.8.1.2.1), the sex ratio (m:f) was lower in the treated groups compared to the control group. Since exposure to abamectin was from days 6-15 of gestation, abamectin could not have affected the sex of the fetuses directly. Apparently, abamectin exposure affects resorption sex-specific (more effect on female fetuses), resulting in a lower m:f ratio, in this study even dose-related.

Increased incidences of exencephaly and cleft palate occurred at dose levels of 0.1 mg/kg bw/day and higher, but there was no correlation between incidence and dose level. The similarity of malformations occurring in the treated groups indicated treatment-related teratogenicity at dose levels of 0.1 mg/kg bw/day and higher.

Acceptability

The study is acceptable as investigative study.

Conclusions

Based on the occurrence of maternal death at levels of 0.5 mg/kg bw/day and higher, the NOAEL for maternal toxicity for the 8,9-Z isomer of avermectin B1a in CF-1 mice in this study is 0.1 mg/kg bw/day.

The 8,9-Z isomer of avermectin B1a is teratogenic in the CF-1 mouse at dose levels of 0.1 mg/kg bw/day and higher, based on excess incidences of cleft palate and exencephaly. Based on these effects, the NOAEL for teratogenicity is 0.05 mg/kg bw/day.

Reliability Indicator	1	
Data Protection Claim	Yes	

98/8 Doc IIIA 6.8.1/ 07 Reproductive Toxicity – Tests on developmental toxicity section No.	**Official use only**

91/414 Annex II Point addressed 5.8.1	Oral teratogenicity	

Title:	L-652,280 oral developmental toxicity study in CF-1 mice	
Lab Report Number:	TT 95-741-0	
Authors:	[redacted] (1996a)	
Test Substance:	L-652,280, abamectin technical (batch no. [redacted], purity [redacted]	
Species:	Mice	
Guidelines:	The method employed for the developmental toxicity element of this study conforms to OECD draft guideline 414 (August 1999) and Council Directive 88/302/EEC, B.31, with the following exceptions: Recording of clinical observations not performed daily throughout gestation No statistical analysis of incidences of malformations and variations Deviations from OECD 414: Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Treatment continued up to day 15 of gestation, cesarian sections performed on day 18	
Date of Report:	31 May 1996	
Published:	No	
GLP:	Yes	

In DAR : STUDY 5 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1996a)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral developmental toxicity study in CF-1 mice.	Doses	:	Insensitive mice: 0, 0.5, 1.0 and 1.5 mg/kg bw/day Sensitive mice: 0 or 0.2-1.0 mg/kg bw/day[2]
Year of execution	:	1995	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a (purity [redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 414 (draft)
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	25 mated females/dose	$NOAEL_{maternal, insensitive mice}$	:	1.5 mg/kg bw/ day
			$NOAEL_{maternal, sensitive mice}$	:	<0.2-1.0 mg/kg bw/day
			$NOAEL_{fetal (sensitive mice)}$	:	could not be established
			$NOAEL_{fetal (insensitive mice)}$	:	<0.5 mg/kg bw/day

1: day 0 of gestation = day vaginal plug observed

2: dose levels increased to 0.3 mg/kg bw/day after 1-3 doses. Two days later, dose level increased to 0.5 mg/kg bw/day. One day later, dose level increased to 1.0 mg/kg bw/day for one day. Dosing then suspended for 2 days and recommenced at 0.75 mg.kg bw/day in 6/18 females of normal appearance. Twelve animals with adverse clinical signs were killed, examined for pregnancy status and discarded.

Study design

According to OECD guideline 414 (draft), with the following deviations: recording of clinical observations not performed daily throughout gestation, no statistical analysis of incidences of malformations and variations, non-gravid uteri not stained for occult implantation sites, uterus/cervix not weighed, treatment continued up to day 15 of gestation, cesarian sections performed on day 18.

Sub-populations of mice sensitive and insensitive to the tremor-inducing property of abamectin were identified, by means of a single gavage dose of 0.4 mg/kg bw abamectin technical. Following preliminary identification of insensitive individuals, the sub-population was treated with a further oral dose of 0.8 mg/kg bw abamectin to confirm their insensitive status. The insensitive animals, and the surviving sensitive animals, were naturally mated 2 - 3 weeks later for a developmental toxicity study. All animals that died or were killed were discarded without necropsy.

Four groups of 25 naturally-mated insensitive female mice were treated orally, by gavage, with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0 (vehicle only), 0.5, 1.0 and 1.5 mg/kg bw/day from day 6 to day 15 of gestation. Two further groups of sensitive mice were similarly treated with 0 (4 animals) or 0.2 - 1.0 mg/kg bw/day (18 animals; see information on doses above). Clinical signs were recorded on day 0 and day 6 to 18 of gestation. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 18 of gestation and food consumption was measured at 3-day intervals from day 3 of gestation. Surviving animals were killed on day 18 of gestation and subjected to a gross necropsy examination of the thoracic and abdominal cavities. Gross lesions were preserved for possible histological examination. The brains of all surviving sensitive animals and of 7 insensitive vehicle control animals and 11 insensitive

animals treated at 1.5 mg/kg bw/day were processed for P-glycoprotein immunohistochemistry. In addition, one-half of each brain from the sensitive animals was also submitted to western immunoblot analysis of P-glycoprotein. The uterus was examined to determine pregnancy status and corpora lutea were enumerated. Implantations were counted and classified as resorptions, dead foetuses or live foetuses. All foetuses were examined externally, weighed and sexed. Placentae were examined for gross changes. Visceral examination by dissection was performed on approximately one-half of the fetuses in each litter and on all externally abnormal foetuses. The heads of these foetuses were fixed for subsequent examination, and all foetuses were examined for skeletal abnormalities and variations.

Results

Results of the study are summarized in table below.

Results of oral developmental toxicity study in CF-1 mice.

Dose (mg/kg bw/day)	**Insensitive**				**Sensitive**	
	0	**0.5**	**1.0**	**1.5**	**0**	**0.2 - 1.0**
Weight gain (g) day 6 - 16	16.6	15.9	16.7	15.8	14.7	4.1
No. pregnant / no. mated	23 / 25	24 / 25	23 / 25	25 / 25	4 / 4	18 / 18
No. examined with live litter	22	24	23	25	4	1
Resorbed or dead litter	0	0	0	0	0	3
No. died / sacrificed	1	0	0	0	0	14
Mean no. corpora lutea/female	13.8	14.0	13.9	14.2	14.5	13.5
Mean no. implantations / female	13.3	13.5	13.6	13.5	11.8	13.5
Mean pre-implantation loss/litter (%)	5.7	5.4	4.4	5.4	20.9	0.0
% resorptions/implantation	5.7	7.6	5.6	8.6	13.6	13.4
% dead foetuses/implantation	0.6	1.0	0.0	0.4	0.0	61.6
% post-implantation loss	6.3	8.6	5.6	8.9	13.6	75.0
No. live foetuses/litter	12.4	12.3	12.8	12.3	10.8	11.0
Sex ratio (M:F)	1 : 0.94	1 : 0.93	1 : 0.91	1 : 1.01	1 : 0.79	1 : 0.57
Mean live male foetal weight (g)	1.21	1.21	1.27	1.22	1.27	1.26
Mean live female foetal weight (g)	1.21	1.19	1.25	1.20	1.30	1.22
External examination:						
No. foetuses (litters) examined	273 (22)	295 (24)	294 (23)	307 (25)	43 (4)	11 (1)
No. foetuses (litters) with malformations	12 (6)	14 (7)	22 (8)	64 (14)	0 (0)	5 (1)
No. foetuses (litters) with variations	14 (8)	26 (8)	33 (14)	45 (16)	5 (3)	0 (0)