	Insensitive				Sensitive	
No. with cleft palate[a]	7	13	21	61	0	5
No. with tail malformation[a]	0	0	0	2	0	0
No. with postaxial pseudopolydactyly[v]	14	26	33	45	5	0
Visceral examination:	No toxicologically relevant effects					
Skeletal examination (body):						
No. foetuses (litters) examined	273 (22)	295 (24)	294 (23)	307 (25)	43 (4)	11 (1)
Dead foetuses (litters) examined	No toxicologically relevant effects					
No. foetuses (litters) with malformations	No toxicologically relevant effects					
No. foetuses (litters) with variations	42 (16)	56 (18)	43 (18)	66 (23)	12 (4)	4 (1)
Skeletal examination (head):						
No. foetuses (litters) examined	131 (22)	142 (24)	142 (23)	143 (25)	21 (4)	5 (1)
No. with cervical rib[v]	20	29	26	24	3	2
No. with supernumerary rib[v]	21	29	17	36	9	2
No. with sternebral variation[v]	4	2	3	11	0	0

[a] malformation

[v] variation

In the preliminary sensitivity-testing phase, 27% of individuals tested displayed clinical signs of neurotoxicity comprising tremors or recumbency. Subsequently, 69% of the sensitive animals died or were killed in extremis, leaving 23 sensitive survivors. The survivors displayed clinical signs for up to 4 days, but were of normal appearance thereafter. Twenty-two animals mated, of which 4 were allocated to the vehicle control group and 18 allocated to be treated at 0.2 - 1.0 mg/kg bw/day. One hundred insensitive females were allocated to the study and mated. Treatment-related deaths and clinical signs were confined to the sensitive group treated at 0.2 - 1.0 mg/kg bw/day. Twelve animals were killed and discarded on days 9-15 of gestation after establishing pregnancy status. A further animal in this group died and another was killed in extremis on day 17. A vehicle control insensitive animal died of apparent dose maladministration. Clinical signs were apparent when the dose level administered was increased to 1.0 mg/kg bw/day.

Four animals survived to day 18, but 3 were recumbent for at least 2 days before necropsy. Treatment-related effects on maternal body weight gain and food consumption were confined to the sensitive group treated at 0.2 - 1.0 mg/kg bw/day. A decrease in weight gain of 74.2% occurred from day 6 to 16, resulting in a group mean body weight of 39.0 g compared with the vehicle control sensitive group value of 53.3 g. Food consumption was markedly reduced from day 11 of gestation. Decreases of 42 % and 83% occurred on days 11 and 17 of gestation, respectively, compared to the vehicle control sensitive group. There were

no treatment-related gross changes in maternal animals at any dose level. Three of the 4 sensitive animals treated at 0.2 - 1.0 mg/kg bw/day produced dead foetuses only. Remarkable are the high incidences of mean pre-implantation loss/litter and % resorptions/implantation in the control sensitive group. In the treated sensitive group, the % dead fetuses/implantation and the % post-implantation loss were 61.6% and 75%, respectively. The sex ratio was lower in the treated sensitve group. Since exposure to the test substance was from days 6-15 of gestation, the test substance could not have affected the sex of the fetuses directly. Apparently, exposure affects resorption sex-specific (more effect on female fetuses), resulting in a lower m:f ratio.

In the insensitive groups, the % resorptions/implantation and the % post-implantation loss were slightly increased in dose groups 0.5 and 1.5 mg/kg bw/day. There was a treatment-related increase in the incidence of cleft palate in the insensitive groups at all dose levels. The effect was dose-related and affected 4.4, 7.1 and 19.9% of foetuses, in order of ascending dose level, compared with a control incidence of 2.6%. A high incidence of cleft palate (45.5%) occurred in the single litter from the sensitive animal treated at 0.2 - 1.0 mg/kg bw/day. The incidence of the variation postaxial pseudopolydactyly was increased in all treated insensitive groups (8.8 - 14.7%) compared to a control incidence of 5.1%.

Minimal to slight diffuse P-glycoprotein staining on the endothelial cell surface of cerebral and cerebellar capillaries occurred in all insensitive female mice. In contrast, no P-glycoprotein expression occurred on the luminal surface of endothelial cells of sensitive female mice.

Acceptability

The study is considered acceptable as investigative study.

Conclusions

The developmental toxicity of the 8,9-Z isomer of avermectin B1a to a sub-population of CF-1 mice sensitive to the tremor-inducing property of abamectin could not be evaluated since only a single viable litter was produced. However, developmental toxicity, characterised by excess incidences of cleft palate, was produced by the 8,9-Z isomer of avermectin B1a in apparently insensitive CF-1 mice at dose levels of 0.5, 1.0 and 1.5 mg/kg bw/day. Therefore, a NOAEL for developmental toxicity could not be established, and the LOAEL in this study for insensitive mice is 0.5 mg/kg bw/day. The isomer did not produce maternal toxicity at dose levels up to 1.5 mg/kg bw/day in the insensitive group, and therefore the NOAEL for maternal toxixcity in insensitive mice is 1.5 mg/kg bw/day. In the sensitive group, body weight gain was decreased during day 6-16 of gestation, and a NOAEL for maternal toxicity could not be established for the sensitive group. Therefore, the LOAEL for maternal toxicity for sensitive CF-1 mice in this study is 0.2-1.0 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	The developmental toxicity of the 8,9-Z isomer of avermectin B_{1a} to a sub-population of CF-1 mice sensitive to the tremor-inducing property of abamectin could not be evaluated since only a single viable litter was produced. However, developmental toxicity, characterised by excess incidences of cleft palate, was produced by the 8,9-Z isomer of avermectin B_{1a} in apparently insensitive CF-1 mice at dose levels of 0.5, 1.0 and 1.5mg/kg bw/day. Therefore, an NOEL for developmental toxicity could not be established. The isomer did not produce maternal toxicity at dose levels up to 1.5mg/kg bw/day.	

98/8 Doc IIIA 6.8.1/ 08 section No.	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex II Point addressed 5.8.1	Oral teratogenicity	

Title:	L-652,280 exploratory oral developmental toxicity study in CF-1 mice of known P-glycoprotein genotype	
Lab Report Number:	TT 96-721-0	
Authors:	[redacted] (1996a)	
Test Substance:	L-652,280 the 8,9-Z photoisomer of avermectin B_{1a} (batch no. [redacted], purity [redacted]	
Species:	Mice, strain Crl:CF-1 [BR	
Guidelines:	Not applicable (investigative study)	
Date of Report:	12 November 1996	
Published:	No	
GLP:	Yes	

In DAR : STUDY 6 (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	:	Exploratory oral developmental toxicity study in CF-mice of known P-glycoprotein genotype.	Doses	:	0 and 1.5 mg/kg bw/day
Year of execution	:	1996	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a [redacted]	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	None (investigative study)
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	12 mated females/dose	NOAEL maternal	:	< 1.5 mg/kg bw/ day
			NOAEL developm	:	< 1.5 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

Study design

Prior to initiation of the study, male and female mice (strain Crl:CF-1 (BR)) were submitted for restriction fragment length polymorphism (RFLP) Southern blot analysis to determine the genotype for the mdr-1 gene (encoding for P-glycoproteins). The females were mated with sexually mature males, to provide groups of 12 mated females producing foetuses of genotypes predictable by the principles of Mendelian inheritance, according to the following schedule:

Treatment	Parental genotype		Predicted foetal genotypes (%)
	Female	Male	(+ / + : + / - : - / -)
0 mg/kg bw/day (vehicle control)	+ / -	+ / -	25 : 50 : 25
	- / -	- / -	0 : 0 : 100
1.5 mg/kg bw/day 8,9-Z isomer	+ / +	+ / +	100 : 0 : 0
	+ / -	+ / +	50 : 50 : 0
	+ / -	- / -	0 : 50 : 50

The 5 groups of 12 mated females were treated orally, by gavage, with the 8,9-Z photoisomer of avermectin B1a in sesame oil at dose levels of 0 (vehicle only) or 1.5 mg/kg bw/day from day 6 to day 15 of gestation. Clinical signs were recorded daily on day 0 of gestation and from day 6 to 18 of gestation.

Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 18 of gestation. Surviving animals were killed on day 18 of gestation and the uterus examined to determine pregnancy status. A distal tail segment was retained from each maternal animal for possible re-analysis of P-glycoprotein genotype. Implantations were counted and classified as resorptions, live or dead foetuses. All foetuses were weighed and examined for external malformations including the palate. One hindlimb of each foetus was removed for possible analysis of P-glycoprotein genotype. At least 4 litters/group were genotyped using DNA isolated from the hindlimb. Placentae from one vehicle control (-/-), one 1.5 mg/kg bw/day female (+/+) and 2 vehicle control (+/-) were retained for Western blotting biochemical analysis of P-glycoprotein. The heads and placentae of foetuses from 4 vehicle control (+/-) females and 4 females treated at 1.5 mg/kg bw/day (+/- x -/-) were treated for p-glycoprotein immunohistochemistry of brain endothelium, palate epithelium, placental

trophoblasts and placental yolk sac endothelium. (Schinkel et al., 19941). The brain of one female treated at 1.5mg/kg bw/day (+/+) was retained for possible immunohistochemical analysis.

Results

Results of the study are summarized in tables below.

Results of exploratory oral developmental toxicity study in CF-mice of known P-glycoprotein genotype.

Treatment level and genotype (F x M):	**0 mg/kg bw/day**		**1.5 mg/kg bw/day**		
	+/- x +/-	**-/- x -/-**	**+/+ x +/+**	**+/- x +/+**	**+/- x -/-**
Mean weight gain day 6 - 18 (g)	25.9	22.7	22.3	20.5	21.3
Overall pregnancy incidence	8 / 12	9 / 12	12 / 12	12 / 12	12 / 12
Implantations (mean/female)	13.9	12.8	12.2	10.8	11.2
No. resorptions	3	10	5	5	8
No. dead foetuses	none				
% post-implantation loss	2.6	10.4	3.6	3.3	5.4
live foetuses (mean/female)	13.5	11.7	11.8	10.4	10.6
Sex ratio (M : F)	1 : 1.20	1 : 1.02	1 : 0.72	1 : 0.87	1 : 1.19
Mean live foetal weight	No toxicologically relevant effects				
No. foetuses (litters) examined	108 (8)	105 (9)	141 (12)	125 (12)	127 (12)
No. foetuses (litters) with malformations	2 (2)	1 (1)	0 (0)	18 (6)	81 (11)
No. foetuses (litters) with variations	No toxicologically relevant effects				
No. (%*) with cleft palate[a]	1 (0.83)	0	0	18 (12.0)	80 (58.0)
No. (%*) with pseudopolydactyly[v]	9 (8.3)	3 (2.8)	3 (2.1)	1 (2.8)	14 (19.0)
No. litters examined	5	4	4	6	5
No. foetuses examined	66	50	39	72	60

[1] Schinkel, A.H., Smit, J.J.M, van Tellingen, O., Beijnen, J.H., Wagennar, E., van Demepter, L., Mol, C.A.A.M., van der Valk, M.A., Robanus-Maandag, E.C., te Rietele, H.P.J., Berns, A.J.M., Borst, P. (1994): Disruption of the mouse mdr 1a P-glycoproteien gene leads to a deficiency in the blood-brain barrier and increased sensitivity to drugs, Cell, 77: 491-502.

Treatment level and genotype (F x M):	0 mg/kg bw/day		1.5 mg/kg bw/day		
No. foetuses with cleft palate genotyped	0	0	0	16	43
No. (%) foetuses with -/- genotype	19 (28.8)	50 (100)	0 (0)	0 (0)	31 (51.7)
No. (%) -/- foetuses with cleft palate	-	-	-	-	30 (96.8)
No. (%) foetuses with +/- genotype	32 (48.5)	0 (0)	0 (0)	41 (56.9)	29 (48.3)
No. (%) +/- foetuses with cleft palate		-	-	16 (39.0)	13 (44.8)
No. (%) foetuses with +/+ genotype	15 (22.7)	0 (0)	39 (100)	31 (43.1%)	0 (0)
No. (%) +/+ foetuses with cleft palate	-	-	-	0 (0)	-

[a] malformation

[v] variation

* litter mean incidence

Results of exploratory oral developmental toxicity study in CF-mice of known P-glycoprotein genotype.

Genotype foetus	-/-	-/+	+/+
No. foetuses examined	31	70	70
No. foetuses with cleft palate	30	29	0
% foetuses with cleft palate	97%	41%	0%

No deaths or treatment-related clinical signs and body weight effects occurred during the study. The weight gain from day 6 to 18 of the vehicle control group (+/- x +/-) was 12.4 - 20.8% higher than all other study groups, but all 8,9-Z isomer-treated groups showed comparable weight gains to the second vehicle control (-/- x -/-) group. A lower weight gain in a sensitive control group (compared to an insensitive control group) was also observed in study 5 of this section.

Notable are the increases in resorptions and post-implantation loss in the -/- x-/- (untreated) and +/- x -/- (treated) groups. Since the effects are present in both control and treated animals, these results are probably related to the comparable genotyping of the animals.

The mean number of implantation sites per female and the mean number of live foetuses were lower in the +/- x +/+ and +/- x -/- treated groups. There were increased incidences of cleft palate in 2 of the 3 groups treated at 1.5 mg/kg bw/day. In the +/- female x +/+ male group the litter mean incidence of foetuses with cleft palate was 12.0% and in the +/- female x -/- male group the litter mean incidence of foetuses with cleft palate was 58.0%, compared with litter mean incidences of 0.83 and 0 % in the vehicle control groups (+/- x +/- and -/- x -/-, respectively). The incidence of cleft palate was 0% in the homozygous positive group (+/+ x +/+) treated at 1.5 mg/kg bw/day.

The incidence of postaxial pseudopolydactyly was higher in the +/- x -/- group (litter mean 19.0%) compared with incidences in the vehicle control groups of 2.8 and 8.3%.

Genotyping of fetuses from at least 4 litters/group confirmed the hypothesis that the markers correlating with sensitivity to abamectin follow normal Mendelian inheritance. When homozygous positive males were mated with homozygous positive females, all progeny were homozygous positive (+/+). Similarly, when homozygous negative males were mated with homozygous negative females, all progeny were homozygous negative (-/-). When heterozygous males and females were mated, the litters contained progeny with all predicted genotypes, +/+, +/- and -/-, in the expected ratio of approximately 1:2:1, respectively.

None of the +/+ genotype foetuses derived from treated females in either of the groups in which the genotype occurred had cleft palate, whereas 97% -/- genotype foetuses exposed to the 8,9-Z isomer of avermectin B1a had cleft palate and 41% of the +/- genotype foetuses were affected.

Histopathological evaluation of foetal brain revealed diffuse P-glycoprotein staining on the endothelial cell surface of cerebral and cerebellar capillaries in most of the heterozygous (+/-) and homozygous positive (+/+) foetuses. In addition, minimal to slight P-glycoprotein staining occurred on the surface of trophoblasts in the placental labyrinth and on the apical surface of epithelial cells of the yolk sac in many of the foetuses with these genotypes. In contrast, no P-glycoprotein expression occurred on the luminal surface of endothelial cells, trophoblasts in the placental labyrinth, or yolk sac epithelial cells in most of the homozygous (-/-) fetuses. No P-glycoprotein staining occurred in the epithelial lining of the oral cavity in the palate region of any foetuses in the study. Western blotting analysis of placentae for mdr1a P-glycoprotein confirmed the results of the immunohistochemical analysis; the amount of P-glycoprotein in the placenta varies with foetal genotype. Thus, the highest amounts were detected in the +/+ placentae, lesser amounts in the +/- placentae and were absent in -/- placentae.

Acceptability

The study is acceptable as investigative study.

Conclusions

The dose level of 1.5 mg/kg bw/day of the 8,9-Z isomer of avermectin B1a resulted in decreased weight gain during day 6-18 of gestation in all treated females as well as in untreated -/- females (mated with -/- males). Since the genotyping of the treated groups more resemble the heterozygous control group than the homozygous negative control group, the decreased weight gain is considered an effect. Therefore, a NOAEL for maternal toxicity could not be established in this study, and the LOAEL is 1.5 mg/kg bw/day.
The dose level of 1.5 mg/kg bw/day of the 8,9-Z isomer of avermectin B1a elicits embryotoxicity, manifested as increased incidences of cleft palate in sensitive foetuses. Foetal sensitivity to the induction of cleft palate is influenced by genotype for the mdr-1 gene encoding for the P-glycoprotein, a gene governed by Mendelian inheritance. Foetal genotype for the mdr-1 gene influences the extent of expression of P-glycoprotein in the brain, placental trophoblasts and yolk sac epithelium. The homozygous positive, heterozygous and homozygous negative genotypes express decreasing amounts of P-glycoprotein and there is an inverse relationship to the incidence of cleft palate. The NOAEL for developmental toxicity in this study could not be established, and the LOAEL is 1.5 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	The dose level of 1.5mg/kg bw/day of the 8,9-Z isomer of avermectin B_{1a} is a NOEL for maternal toxicity and reproductive indices, but elicits embryotoxicity, manifested as increased incidences of cleft palate in sensitive foetuses. Foetal sensitivity to the induction of cleft palate is influenced by genotype for the mdr-1 gene encoding for the p-glycoprotein. a gene governed by Mendelian inheritance. Foetal genotype for the mdr-1 gene influences the extent of expression of P-glycoprotein in the brain, placental trophoblasts and yolk sac epithelium. The homozygous positive, heterozygous and homozygous negative genotypes express decreasing amounts of P-glycoprotein and there is an inverse relationship to the incidence of cleft palate.	

98/8 Doc IIIA 6.8.1/ 09 Reproductive Toxicity – Tests on developmental toxicity section No.	**Official use only**

91/414 Annex Point addressed	II 5.8.1	Oral teratogenicity	

Title:	L-652,280 oral developmental toxicity study in CD-1 mice	
Lab Report Number:	TT 96-732-0	
Authors:	[redacted] (1996b)	
Test Substance:	L-652,280, the 8,9-Z isomer of avermectin B_{1a} ([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 draft guideline 414: Non-gravid uteri not stained for occult implantation sites Uterus/cervix not weighed Treatment continued up to day 15 of gestation, caesarean sections performed on day 18	
Date of Report:	18 November 1996	
Published:	No	
GLP:	Yes	

In DAR : STUDY 7 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1996b)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral developmental toxicity study in CD-1 mice.	Doses	:	0, 0.75, 1.5 and 3.0 mg/kg bw/day

Year of execution	:	1996	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:CD-1 [BR] strain)	Acceptability	:	Acceptable as investigative study
Group size	:	22 mated females/dose	NOAEL maternal	:	3.0 mg/kg bw/ day
			NOAEL developm	:	< 0.75 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

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.

Four groups of 22 naturally-mated female mice (Crl:CD-1 [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), 0.75, 1.5 and 3.0 mg/kg bw/day from day 6 to day 15 of gestation. Clinical signs were recorded on day 0 of gestation and from 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. The uterus was examined to determine pregnancy status and corpora lutea were enumerated. Implantations were counted and classified as resorptions, dead fetuses or live fetuses. All fetuses 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 fetuses. The heads of these fetuses were fixed for subsequent examination. All fetuses were examined for skeletal abnormalities and variations.

Results

There were no deaths and no treatment related clinical signs during the study. There were no treatment related effects on maternal bodyweight gain or food consumption. There were no treatment related gross findings at necropsy in maternal animals. Pregnancy indices, embryo survival parameters and foetal weights were unaffected by treatment. There was an increased incidence in cleft palate (9.5%, 5% and 13.6% in the treated groups versus 0% in the controls). Although there is no dose relationship, the increased incidences are considered treatment related. There were slight increases in the incidence of malformations and variations, with highest incidences in the highest dose group (see table).

Dose (mg/kg bw/day)	0	0.75	1.5	3.0
Cleft palate (% of litters)	0	9.5	5	13.6

Fetuses with malformation (%)	0	5	3	6
Litters with malformation (%)	0	24	10	23
Fetuses with variation (%)	2	2	4	12
Litters with variation (%)	10	5	10	18

Conclusion

The NOAEL of the 8,9-Z isomer of avermectin B1a for maternal toxicity in CD-1 mice in his study is 3.0 mg/kg bw/day, since there was no evidence of maternal toxicity in the highest dose group of 3.0 mg/kg bw/ day. Based on the increased incidence of cleft palate in all treated groups, an effect which is considered induced by the substance and is observed in several studies, A NOAEL for developmental toxicity could not be derived, and the LOAEL in this study is 0.75 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	There was no evidence of maternal toxicity or developmental toxicity up to 3.0 mg/kg bw/ day of the 8,9-Z isomer of avermectin B_{1a} when administered to CD-1 mice. Thus the NOELs for both maternal and developmental toxicity are 3.0 mg/kg bw/day.	

98/8 Doc IIIA 6.8.1/ 10 section No.	Reproductive Toxicity – Tests on developmental toxicity	Official use only
91/414 Annex II Point addressed 5.8.1	Ten day maternotoxicity study in mice	

Title:	Ten day dietary maternotoxicity study in mice	
Lab Report Number:	TT 83-705-1	
Authors:	[redacted] (1984g)	
Test Substance:	tritiated and unlabelled abamectin technical MK-0936, batch nos. [redacted], purity [redacted], respectively	
Species:	Mice	
Guidelines:	Not applicable (investigative study)	
Date of Report:	23 March 1984 (revised 11 April 1984)	
Published:	No	
GLP:	Yes	

In DAR : STUDY 1 (6.8 Further toxicological studies/6.8.2 Supplementary studies)

Characteristics

Reference/notifier	:	[redacted] (1984g)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Ten day dietary maternotoxicity study in mice.	Doses[2]	:	0, 0.1, 0.3 and 0.6 mg/kg bw/day (time-weighted average 0, 0.08, 0.24 and 0.48 mg/kg bw/day)
Year of execution	:	1983	Vehicle	:	acetone
Test substances	:	Tritiated abamectin technical (<0.5%, purity [redacted]) and unlabelled abamectin technical (MK-0936, purity [redacted]	GLP statement	:	yes
Route	:	Oral (diet)	Guideline	:	Not applicable, investigative study
Species	:	Mice (albino CF-1 strain)	Acceptability	:	Acceptable as investigative study
Group size	:	20 mated females/dose	NOAEL maternal	:	0.08 mg/kg bw/ day

1: day 0 = day of vaginal plug observed.

2: The mean achieved dose levels from day 6 to 11 were 0.10, 0.33 and 0.61 mg/kg bw/day abamectin technical and from day 12 to 16 were 0.06, 0.16 and 0.33 mg/kg bw/day, in order of ascending dose level.

Study design

Four groups of 20 naturally-mated female mice were treated orally with a mixture of tritiated (<0.5% total) and unlabelled abamectin technical, by admixture in the diet at constant concentrations of 0 (vehicle only), 0.33, 1.0 and 2.0 ppm from day 6 to day 15 of gestation (day 0 = day vaginal plug observed). Target dose levels were 0, 0.1, 0.3 and 0.6 mg/kg bw/day, which were achieved on days 6 to 11. However, on days 12 to 16 of gestation, due to lower food consumption, achieved dose levels reduced to 0.06, 0.16 and 0.33 mg/kg bw/day, respectively. Clinical signs were recorded daily from day 6 to day 17 of gestation. Body weights were recorded on days 0, 6, 11, 16 and 17 of gestation and food consumption was measured. The animals were killed on day 17 of gestation and the unopened uterus was examined to estimate the numbers of implantation sites, resorptions and live foetuses. Dead foetuses were classified as resorptions.

Results

Two females treated at 0.6 mg/kg bw/day were killed in poor condition after 3 days of treatment having developed marked tremors. Three females treated at 0.3 mg/kg bw/day showed hunched posture and tremors after 6 or 7 days of treatment and were also killed in poor condition. There were no clinical signs or animals killed prematurely at 0.1mg/kg bw/day. From day 12 to 16, food consumption was decreased in the 0.3 and 0.6 mg/kg bw/day groups.The mean weight gains of all treated groups were significantly higher than the controls from day 16 to 17, resulting in a treatment-related increase in terminal body weights of 3.1 to 5.5% . There were no treatment-related effects on reproductive status, as assessed by pregnancy incidence, number of implantations and the numbers of live foetuses and resorptions.

Acceptability

The study is considered acceptable as investigative study.

Conclusions

The NOAEL for maternal toxicity for abamectin technical in this study is established as 0.08 mg/kg bw/day (time-weighted average), based on the occurence of poor condition and hunched posture and/or tremors at dose levels of 0.3 and 0.6 mg/kg bw/day.

SYNGENTA CONCLUSIONS

Conclusion:	A NOAEL for maternal toxicity for abamectin technical was established as 0.1mg/kg bw/day (time-weighted average	

	0.08mg/kg bw/day) by incorporation into diet, based on the occurrence of poor condition and hunched posture and/or tremors at dose levels of 0.3 and 0.6 mg/kg bw/day. A NOEL for reproductive parameters was established as > 0.6mg/kg bw/day (time-weighted average >0.48mg/kg bw/day), based on no effects on pregnancy incidence, number of implantations and foetal viability at the highest dose level tested.	

98/8 Doc IIIA 6.8.1/11 section No.	P-glycoprotein expression study in rats Tests on developmental toxicity	Official use only
91/414 Annex II Point addressed 5.8.1	Exploratory study of P-glycoprotein development in rat pups	

Title:	Exploratory Study of P-Glycoprotein Development in Rat Fetuses and Pups. Exploratory P-glycoprotein Development in rat foetuses and pups. Addendum to unpublished report No. TT #94-739-0	
Lab Report Number:	TT 94-739-0; 3 March 1995 Addendum	
Authors:	[redacted] (1995). [redacted] (1996b) (Addendum)	
Test Substance:	Not specified	
Species:	Rat	
Guidelines:	Not applicable (investigative study)	
Date of Report:	3 March 1995 Addendum:	
Published:	No	
GLP:	No	

In DAR : STUDY 6 (6.8 Further toxicological studies/6.8.2 Supplementary studies)

STUDY 6

Characteristics

Reference/notifier	:	[redacted] (1995); [redacted] (1996b) (addendum)	Exposure	:	-
Type of study	:	Exploratory study of P-glycoprotein development in rat fetuses and pups.	Doses	:	-
Year of execution	:	1995-1996	Vehicle	:	-
Test substances	:	none	GLP statement	:	no
Route	:	-	Guideline	:	Not applicable (exploratory study)
Species	:	Rat (Sprague-Dawley Crl:CD (SD)	Acceptability	:	acceptable

		BR
Group size	:	40 mated and 4 unmated F0 females; F1: 4 pups/sex/day

Study design

The purpose of this study was to determine levels of P-glycoprotein immunohistochemically and by Western blot analysis in the brain and intestine of adult rats, gestation day 20 rat fetuses, and rat pups on postnatal days 2, 5, 8, 11, 14, 17 and 20. There were 40 mated F0 females and 4 unmated females assigned to this study, none were treated with a test or control substance. During cohabitation, females were housed with untreated males of the same strain in a ratio of 1:1. Mating was confirmed by the presence of seminal plug(s) in the cage pan and/or vagina. Mortality of F0 females was checked daily. Body weights and food consumption were not recorded. Scheduled live foetuses from F0 females were weighed, sexed and given external examinations. Selected F1 offspring were sexed and externally examined on postnatal day 0 and weighed at sacrifice.

Foetal Analyses: Four F0 females were sacrificed on gestation day 20. Within each litter, one male and one female foetus were terminated and the brain and jejunum were prepared for immunohistochemical analysis for P-glycoprotein. In addition, samples of brain and jejunum were stored and later analyzed for P-glycoprotein by Western blot analysis (see addendum). The brain, jejunum and uterus of the four dams were sampled, sectioned, and analyzed as above by immunohistochemical technique and by Western blot technique.

F1 offspring: On Postnatal Days 2, 5, 8, 11, 14, 17 and 20, one male and one female pup from each of four litters (total of four pups/sex/day) were sacrificed and sampled and sectioned as described above for immunohistochemical analysis of P-glycoprotein in the brain and jejunum. Selected samples were analyzed for P-glycoprotein by Western blot analysis.

Non-pregnant females: Four adult non-pregnant females were sacrificed and the uteri were sampled, sectioned and analyzed as decribed above for pregnant females. The frozen sections were processed immunohistochemically using methods adapted from those of Schinkel et al. (1994) for P-glycoprotein, and slides were examined microscopically.

Addendum: At a later date, Western blots produced in the study were analysed using densitometry of the P-glycoprotein bands to quantify the levels of protein.

Results

There were no deaths or abortions during the study. External examination of foetuses and pups did not reveal any anomalies.

P-glycoprotein staining was observed in a diffuse pattern on the endothelial cell surface of capillaries in the cerebrum and cerebellum and the brush border of jejunal epithelial cell. In contrast to the non-pregnant rats

which had no uterine P-glycoprotein expression, the pregnant rats had a moderate amount of P-glycoprotein present on the luminal surface of the uterine epithelium. The P-glycoprotein observed microscopically in the brain and uterus was confirmed by Western Blot analysis. F1 pups of all ages, including the gestation day 20 foetuses, showed P-glycoprotein staining in the brain. Expression appeared weaker in the gestation day 20 foetuses and younger pups compared to the older postnatal day 17 and 20 pups.
P-glycoprotein staining of the jejunal epithelial cells was not apparent until approximately postnatal day 8. P-glycoprotein staining appeared more intense in the older pups by approximately postnatal day 17 but even by postnatal day 20 did not achieve the intensity found in the F0 adult females. The intensity of the P-glycoprotein seen microscopically in the F1 pups was further supported by Western Blot analysis.
Addendum: Quantitation by scanning densitometry of Western blots of brain samples shows that levels of P-glycoprotein in the gestation day 20 foetus are very low (approximately 11% of the adult value), as are values for neonatal rats through to postnatal day 11 (see table). It is suggested by the study author, that the apparent increase in the value for the rat foetus (11%) compared to the early post-natal samples (5%) is probably a reflection of qualitative differences of the protein as a function of age. The Western blots showed that the rat foetal protein migrates in a more diffuse pattern compared to the neonatal samples. Since this protein undergoes significant post-translational processing i.e. glycosylation, it is possible that the foetal protein is not a fully mature, functional protein equivalent to that in older animals. However, it is clear that the neonatal animals through to postnatal day 14 have significantly lower levels of P-glycoprotein in the brain compared to older neonates and adults. These differences in P-glycoprotein correlate with the increased neonatal toxicity to the avermectins observed from approximately day 4 through to day 14 in multigeneration studies.

Scanning densitometry of Western blots showing rat P-glycoprotein expression levels at various stages of development

Animal Status	Signal volume (P-gp)	% of adult level
F_0 Females Pregnant	641.8	100.0
F_1 Pups Gestation Day 20	72.36	11.3
F_1 Pups Postnatal Day 2	41.82	6.5
F_1 Pups Postnatal Day 5	36.68	5.7
F_1 Pups Postnatal Day 8	28.08	4.4
F_1 Pups Postnatal Day 11	44.49	6.9
F_1 Pups Postnatal Day 14	122.9	19.1
F_1 Pups Postnatal Day 17	239.5	37.3

F_1 Pups Postnatal Day 20	571.3	89.0

P-gp: P-glycoprotein

Acceptability

The study is considered acceptable as exploratory study.

Conclusions

It was demonstrated that the expression of P-glycoprotein on the luminal surface of endothelial cells of cerebral and cerebellar capillaries (and tight-junctions of these endothelial cells) is lower in neonate rats compared to adult rats. The expression of P-glycoprotein develops to full (adult) extent during the first 20 days. Furthermore the expression of P-glycoprotein in the jejunal epithelial brush border does not start before postnatal day 8. It is suggested that neonate rats, with limited or no P-glycoprotein expression have increased susceptibility to avermectin toxicity.

98/8 Doc IIIA 6.8.1/12 section No.	P-glycoprotein expression study in rats Tests on developmental toxicity	Official use only
91/414 Annex II Point addressed 5.8.1/ 04	Examination of developmental expression of P-glycoprotein levels in rat pups	

Title:	Developmental Expression of P-Glycoprotein (Multidrug Resistance Gene Product) in the Rat Brain	
Lab Report Number:	Journal of Neurobiology, Vol. 39 (3), 383-392	
Authors:	[redacted] (1999).	
Test Substance:		
Species:	Rat	
Guidelines:	Not applicable (investigative study)	
Date of Report:	1999	
Published:	Yes	
GLP:	No	

In DAR : STUDY 7 (6.8 Further toxicological studies/6.8.2 Supplementary studies)

Characteristics

Reference/notifier	:	[redacted] (1999)	Exposure	:	-
Type of study	:	Examination of developmental expression of P-glycoprotein levels in rat pups.	Doses	:	-
Year of execution	:	unknown	Vehicle	:	-
Test substances	:	none	GLP statement	:	no
Route	:	-	Guideline	:	Not applicable (exploratory study)
Species	:	Rat (Crj:Wistar)	Acceptability	:	acceptable
Group size	:	5/time period			

Study design

The purpose of this study was to examine the expression and localization of P-glycoprotein in the brain of adult female rats and neonate rat pups during their early development.

Rats pregnant for 19 days were anesthetized and rat fetuses were removed from the uteri by cesarean section. Rats, postnatal days 1, 3, 7, 14, 21, 28, 56 and 84, were anesthetized and the brains were dissected. Five rats were used at each time period. Brains were homogenized and separated by centrifugation into a cytosolic fraction and a membrane fraction. Aliquots were separated by SDS-PAGE electrophoresis and P-glycoprotein was marked by a specific antibody, and quantitatively analysed.

Immunohistochemistry was performed on brain slices to determine the localization of P-glycoprotein in relation to brain capillaries and astrocytes.

Results

In the adult rat brain (postnatal day 84), P-glycoprotein was detected in the membrane fraction of the cerebral cortex, cerebellum and the hippocampus, predominantly in the membrane fraction.

Developmental changes in P-glycoprotein expression: P-glycoprotein was first detected from postnatal day 7 in the membrane fraction of cerebral cortex, cerebellum and hippocampus, and levels gradually increased, reaching a plateau on postnatal day 28 (cerebral cortex) or postnatal day 50 (cerebellum). The observed expression profile of P-glycoprotein in the membrane fraction was comparable in the cerebral cortex, hippocampus and cerebellum.

Localisation of P-glycoprotein in the rat brain: Immunolocalisation of P-glycoprotein in brain slices confirmed that P-glycoprotein was not detectable until postnatal day 7. Intense staining was seen on postnatal day 21. Double immunostaining of P-glycoprotein with von Willebrand factor (a marker for capillaries) and glial fibrillary acidic protein (GFAP – a marker for astrocytes) showed that P-glycoprotein was colocalised with the capillaries but not the astrocytes.

Acceptability

The study is considered acceptable as exploratory study.

Conclusions

Adult rats show intense P-glycoprotein immunoreactivity. P-glycoprotein was undetectable in the embryo and early stages of postnatal development. It was first detected on postnatal day 7 and then gradually increased to reach a plateau at levels approximating to those seen in adult rats. There is evidence that P-glycoprotein expression is localized in the brain capillaries (suggesting a role for P-glycoprotein in the blood brain barrier).

98/8 Doc IIIA 6.8.1/13 section No.	**P-glycoprotein expression study in mice** **Tests on developmental toxicity**	**Official use only**
91/414 Annex II Point addressed 5.8.2	Oral teratogenicity	

Title:	Abamectin: Exploratory acute oral toxicity study in mice	
Lab Report Number:	TT 96-2727	
Authors:	[redacted] (1997)	
This study summary is presented at '1. Acute Toxicity'; 6.1.1/07 Acute toxicity – Oral In DAR : STUDY 4 (6.8 Further toxicological studies/6.8.2 Supplementary studies)		

98/8 Doc IIIA 6.8.1/14 section No.	**Exploratory 5 day oral mouse** **Tests on developmental toxicity**	**Official use only**
91/414 Annex II Point addressed 5.8.2	Oral teratogenicity	

Title:	Exploratory 5-day oral toxicity study comparing abamectin sensitivity and P-glycoprotein levels in CF-1 and CD-1 mice	
Lab Report Number:	TT 94-2775	
Authors:	[redacted] (1994)	
Test Substance:	Abamectin (batch no. [redacted], purity [redacted]	
Species:	CF-1 Mice	
Guidelines:	Not applicable (investigative study)	

Date of Report:	12 September 1994	
Published:	No	
GLP:	No	

In DAR : **STUDY 5** (6.8 Further toxicological studies/6.8.2 Supplementary studies)

Characteristics

Reference/notifier	:	[redacted] (1994)	Exposure	:	4 days
Type of study	:	Comparative 5-day oral toxicity study in CF-1 and CD-1 mice	Doses	:	0.8 mg/kg bw/day
Year of execution	:	unknown	Vehicle	:	sesame oil
Test substances	:	Abamectin (purity [redacted])	GLP statement	:	no
Route	:	Oral (gavage)	Guideline	:	Not applicable (in house investigative study)
Species	:	Mice (CF-1 strain and CD-1 strain)	Acceptability	:	acceptable
Group size	:	49m+50f CF-1 strain and 4/sex CD-1, 5/sex/strain control mice			

Study design

It is suggested, that a deficiency in P-glycoprotein levels in brain capillary endothelial cells which form the blood-brain barrier would result in enhanced sensitivity to abamectin-induced neurotoxicity in CF-1 mice compared to CD-1 mice. This study was performed to investigate the P-glycoprotein levels in brain and small intestine in correlation with sensitivity to the neurotoxic effects of low doses of abamectin in CF-1 and CD-1 mice.

To identify abamectin-"sensitive" and "-insensitive" CF-1 mice, two groups of mice (49m+50f CF-1 strain mice and 5m+5f CD-1 strain mice), were treated orally, by gavage, with 0.8 mg/kg bw/day abamectin as a solution in sesame oil, for 4 days. Two further groups, (5m+5f CF-1 strain mice and 5m+5f CD-1 strain mice), received sesame oil for 4 days, as vehicle control groups. All animals were weighed pre-dose and observed for signs of neurotoxicity several times each day. Animals which showed severe tremors and/or ataxia at this dose level were killed and exsanguinated. A total of 3 vehicle control mice (1 male and 2 females) of the same strain were killed and exsanguinated at the same time. All remaining vehicle control animals and 20 randomly selected CF-1 strain "insensitive" mice were killed on day 4. Brain and small intestine were removed at necropsy. Sections of cerebral cortex, cerebellum and jejunum were frozen for immunohistochemical analysis of P-glycoprotein by an adaption of a published method (Schinkel *et al.*, 1994), and examined for relative staining intensity of P-glycoprotein against control sections of each tissue. Further samples of brain (cerebrum and cerebellum) and small intestine were prepared for subsequent SDS-PAGE western immunoblot analysis of P-glycoprotein content. Crude membrane protein fractions, as the source of P-glycoprotein, were prepared by a modification of the method of [redacted], (1987).

Following completion of the immunohistochemical studies, 5/sex/group insensitive CF-1 strain mice and a random sample of naïve CD-1 strain mice (5/sex or 10 f/group) were treated with single oral doses of abamectin ranging from 1.0 to 10 mg/kg to determine their sensitivity to abamectin for comparison with the sensitive CF-1 sub-group.

Results

In CF-1 mice, treatment with 0.8 mg/kg/day abamectin induced whole body tremors and slight ataxia. The signs first occurred within 1.5 h of treatment on day 1. Within 4 h of treatment, 12 female and 5 male CF-1 mice, 17% of those tested, showed severe signs of toxicity comprising dyspnea, lateral recumbency, tremors and coma following handling. These animals, identified as sensitive to abamectin toxicity, were killed and subjected to necropsy together with 2 females and one male from each of the other groups.

Immunohistochemical analyses of brain and small intestine showed that 11 of the 12 CF-1 strain females and all the CF-1 strain males identified as sensitive had no detectable P-glycoprotein in any of the tissues examined. The other sensitive CF-1 female had a minimal amount of P-glycoprotein in the brain sections. In contrast, the 20 CF-1 strain "insensitive" individuals evaluated for the presence of P-glycoprotein all had detectable P-glycoprotein, from minimal to intense staining, in all tissues examined of both sexes. Similarly, all CD-1 strain mice of both sexes treated with abamectin showed moderate to intense staining in all tissues examined, with the exception of a single female with minimal staining in the jejunum. CD-1 control animals showed comparable staining to the treated animals, indicating that abamectin does not influence P-glycoprotein levels.

The results of the western immunoblot analyses confirmed the results of the immunohistochemical visualisation of P-glycoprotein. CD-1 strain animals subjected to SDS-PAGE showed intense banding of P-glycoprotein in all 3 tissues, whereas the 2 CF-1 strain sensitive animals evaluated showed no detectable P-glycoprotein. Insensitive CF-1 strain mice showed similar levels of P-glycoprotein to CD-1 strain mice.

The CF-1 strain "insensitive" mice and CD-1 strain mice re-challenged with either 1.0 or 2.5 mg/kg abamectin showed no clinical signs of toxicity. Re-challenge with 5.0 or 10.0 mg/kg produced transient slight tremors and ataxia in 6/10 and 8/10 CF-1 strain "insensitive" mice. The effect occurred 5-6 h after treatment, and persisted for approximately 19 h in all animals with the exception of one animal treated at 10.0 mg/kg which continued to show clinical signs for a further 24 h.

Acceptability

The study is considered acceptable as investigative study.

Conclusions

In this study, approximately 17% of a random CF-1 strain mice were sensitive to abamectin toxicity, whereas CD-1 strain mice were insensitive. Sensitivity in CF-1 strain mice was related to the absence of P-glycoprotein in brain and small intestine.

SYNGENTA CONCLUSIONS

Conclusion:	Approximately 17% of a random population of CF-1 strain mice are sensitive to abamectin toxicity but CD-1 strain mice are uniformly insensitive. Sensitivity in CF-1 strain mice is directly related to the absence of p-glycoprotein in brain and small intestine. CF-1 strain mice expressing higher levels of P-glycoprotein are more resistant to abamectin toxicity than P-glycoprotein deficient individuals.	

Additional data notifier (copied from the abamectin PPP DAR)

In 2005 the notifier submitted additional data on reproduction and developmental toxicity. The additional information concerned literature on estrus cycle patterns/reproduction in rats and on expression of P-glycoprotein in mice and rats. Furthermore, historical control data of rats are provided for sex ratio, cleft palate, extra rib and vertebral count variation. Mice historical control values included sex ratio, cleft palate, exencephaly, postaxial pseudopolydactyly, hind limb hyperextension, incomplete ossification of sternabrae, vertebrae and skull and extra rib. All control data were from studies with the 8,9-Z isomer of abamectin B1a. The notifier also provided some overviews of observed effects in several studies.

This additonal information provided by the notifier did not result in changed opinions concerning the effects observed in animals treated with abamectin or the 8,9-Z isomer of abamectin.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12 november 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]

Acceptability	[redacted]
Remarks	[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	

98/8 Doc IIIA section No.	6.8.2/ 01	Reproductive Toxicity – Two Generations	Official use only
91/414 Annex Point addressed	II 5.6.1 / 01	Two Generation reproductive toxicity	

Title:	Reproductive effects of MK-0936 administered orally by gavage to Crl:COBS CD (SD) BR rats for two generations	
Lab Report Number:	No. TT 82-9010	
Authors:	[redacted]	
Test Substance:	Abamectin (MK-0936, batch no. [redacted], purity of batch not reported but specified elsewhere [redacted], 1984g] as [redacted] by HPLC)	
Species:	Rat	
Guidelines:	Test method conforms to, and generally exceeds, OECD guideline no. 416 (May 1983) and 88/302/EEC, B.35, with the following exceptions: Food consumption was not measured. Reproductive organs of animals failing to mate were not examined microscopically. P generation females were not subjected to necropsy Only 10 male and 25 female F1 parental animals were subjected to necropsy	
Date of Report:	15 June 1984	
Published:	No	
GLP:	Yes	

STUDY 1 in DAR (B.6.6.1 Reproductive toxicity), with revisions and additional information in the

revised addendum (Febr. 2008). The text and study summary below are copied from the addendum. The text below is complicated, because the original evaluation of the study in the DAR appeared to be not correct, based on incorrect presentations of the results in the original study report. Below, first, where relevant, an analysis is made of the raw study data and subsequently the study itself is summarized.

The original study report predominantly presented data when an effect was observed in e.g. one generation or in one mating, but did not present all data on all generations or matings. In addition, in the original study report the calculation of the cohabitation times was incorrect. The summary and conclusions in the DAR were based on the information provided in the study report. In view of the new data provided by the notifier it is clear that in the DAR, not the complete picture of the study was presented and observed effects could not be put in perspective. The applicant submitted additional data, derived from the raw data of the study, including an amendment to the original report, prepared by the performing laboratory. Below, the implications of the new data from the applicant are first described, and subsequently, the evaluation of the 2-generation study in rats is copied from the DAR, with amendments where necessary, based on the additional data from the applicant.

Reconsideration of the effects of abamectin on reproduction parameters in a two-generation study of reproductive toxicity in the rat, on the basis of new data.

The effects of abamectin on reproduction were investigated in a two-generation reproductive toxicity study in the rat ([redacted] 1984e). In the DAR of 2005 it was concluded that in this study abamectin induced:

1. increased mating time with 1st male during F1a mating, and increased duration of cohabitation in the F0 generation
2. increased number of F0 dams with prolonged interestrus and
3. decreased number of F0 animals mating for generation of the F1b litter

Based on these effects a classification with either R60 or R62 was proposed.

In the original study report not all individual data were presented, and for some parameters data were lacking. The notifier disagreed with the proposal for classification in the DAR and argued that some of the data/calculations in the original study report were incorrect (see reporting table 2(5)). The notifier provided individual rat data from the reproduction study and recalculated some of the parameters in order to support their arguments.

The present evaluation addresses the arguments of the notifier in the light of the newly provided data,

derived from the raw study data.

Increased duration of cohabitation in F0 generation

The notifier argued that the increase in co-habitation times was not apparent after the data were corrected by the testing facility and therefore there is no treatment related effect on co-habitation times.

Standard practice for calculation of this endpoint requires inclusion of all pairings that produced evidence of mating (sperm present in vaginal smear) but exclusion of pairings that failed to provide such evidence. Failure to follow this approach confounds the interpretation of effects on two different endpoints:

- number of animals mating
- cohabitation time prior to mating (for those animals exhibiting evidence of mating)

In the study report, a footnote to Table 9 indicated that the correct procedure had been followed. However, closer analysis of the data by the notifier and testing facility, together with examination of the relevant raw data, revealed that the correct procedure had not been followed. In contrast, calculation of cohabitation time had included all pairings, regardless of whether evidence of mating was seen. Examination of all raw data revealed errors in the calculation of cohabitation times for all matings. The values presented in the study report were therefore incorrect. The recalculated group mean cohabitation times for the F0/F1a, F0/F1b, F1b/F2a and F1b/F2b matings using the correct procedure are shown in Table below, provided by the notifier.

Corrected group mean cohabitation time values

		0 mg/kg	0.05 mg/kg	0.12 mg/kg	0.4 mg/kg
F0/F1a	**M**	3.7	2.4	3.3	3.7
	F	4.6	3.8	3.3	5.3
F0/F1b	**M**	2.2	3.0	3.6	3.3
	F	3.8	4.3	4.5	5.3
F1b/F2a	**M**	2.8	3.2	3.0	2.6
	F	3.6	4.6	4.3	3.6
F1b/F2b	**M**	4.2	6.2	6.8	2.3
	F	6.9	8.6	9.3	4.7

Correct calculation of these values clearly indicates no consistent effect on cohabitation time in any mating. There is large variability both within and between groups and there were no statistically significant differences between the treated groups and the controls. It is therefore concluded that there is no treatment-

related effect on cohabitation time during any mating. The testing facility which conducted the study has issued an Addendum to the original study report in light of the errors discovered by the notifier.

The RMS confirms that the corrected cohabitation time values provided by the notifier indicate that, with respect to animals that do mate, there is no effect of abamectin on cohabitation time.

Number of F0 dams with prolonged interestrus during F1b mating

In the F0 females there seems to be an increase in the percentage of dams in prolonged estrus (persistent estrus) at 0.4 mg/kg bw/day (13.3%) vs control females (3.6%), suggesting an effect of abamectin. However, the notifier has provided new data that show that in the F1b generation, during mating for the production of the F2b generation, 9 control females (28%) are in persistent estrus and at 0.4 mg/kg bw/day 4 females (13%) are in persistent estrus.

Spontaneous persistent vaginal estrous, a normal acyclic state occurring in older rats, reflects the presence in the ovaries of large vesicular follicles failing to luteinise and, hence, the absence of corpora lutea once the condition becomes well established. Indeed, manifestation of intervals of persistent estrous is one of the earliest signs of reproductive senescence in rats. This normal aging phenomenon has been extensively described in most commonly used laboratory strains of rats ([redacted] 1977; [redacted] 1999). Importantly, it is also well established that in the Sprague Dawley strain (used in the studies discussed here), control of the female estrous cycle begins to decline at a relatively young age in a manner that leads to episodes of persistent estrous [redacted] 1977; [redacted] 1977; [redacted] 1999). Indeed, recent studies have shown that normal control of reproductive estrous cycling in the SD rat strains can destabilise at less than 6 months of age ([redacted] 1999) – see Table below.

Early onset of persistent estrous in control SD rats (taken from [redacted] 1999)

Age of rat (weeks)	Number of animals exhibiting persistent estrous (Total 90 animals at each age range)
7-8	0
11-12	0
15-16	1
19-20	10
23-24	13
27-28	13

31-32	26

It is noteworthy therefore that the F0 and F1b parental females were at least 24 weeks old at the time of mating for generation of the F1b and F2b litters, respectively. The age of these animals was therefore consistent with the age at which SD female rats are known to enter persistent estrous. Estrous cycling measurements were only performed during the cohabitation time and daily measurements throughout the 14-21 day mating period are not available for all animals, since they were only taken up to the point of apparent mating. The data are summarised in Tables below.

F0 parental females exhibiting persistent estrous during F1b mating

	0 mg/kg	**0.05 mg/kg**	**0.12 mg/kg**	**0.4 mg/kg**
Persistent estrous (% total below)	1 (33)	0 (0)	0 (0)	4 (30)
Total animals with 14-21 day estrous cycling data	3	3	3	12
Number mated/cohabited	28/28	30/30	30/30	22/30

F1b parental females exhibiting persistent estrous during F2b mating

	0 mg/kg	**0.05 mg/kg**	**0.12 mg/kg**	**0.4 mg/kg**
Persistent estrous (% total below)	9 (60)	9 (53)	7 (41)	4 (40)
Total animals with 14-21 day estrous cycling data	15	17	17	10
Number mated/cohabited	28/32	27/32	28/32	26/31

Unsurprisingly, evidence of persistent estrous (prolonged estrous stages) is associated with a lower number of matings across all dose groups, including controls.

The F1b/F2b mating data, together with published literature data, provides strong evidence that by around the age of 6 months, SD female rats (control and/or abamectin-treated) have either entered, or are on the threshold of entering, persistent estrous. Although the overall incidence of persistent estrous is lower in the F0/F1b mating, this is because fewer animals were measured; the proportion of high dose animals in persistent estrous was essentially equivalent to the control. It is not possible to determine the prior estrous cycling history of all animals in the study. Therefore, there is a limited data set from which to interpret any effects on estrous cycling. Nevertheless, there is no convincing evidence that F0/F1b mating high dose group had a higher proportion of animals in persistent estrous compared with the controls. The inherent variability of this endpoint assessed at a "snapshot in time", together with no evidence of a treatment-

related effect in the F1b/F2b mating, leads to the conclusion that the above finding reflects a normal age-related phenomenon in female SD rats and is unrelated to administration of abamectin.

Decreased number of F0 animals mating for the F1b generation.

As discussed above there may have been an association between the incidence of persistent estrous and number of animals mating. However, for the reasons stated the limited dataset precludes drawing any definitive conclusions. It is important in examining the F0/F1b mating to look at the reproductive performance across the generations to establish the evidence for the consistency and reproducibility of findings. Key reproductive parameters are summarized for each mating in Table below.

Key reproductive parameters*

Parameter	Parents	Mating	0 mg/kg	0.05 mg/kg	0.12 mg/kg	0.4 mg/kg
Number of males not showing positive indication of mating with the first female	F0	F1a	2	3	1	4
	F1b	F2a	2	4	5	2
	F0	F1b	3	3	2	**11**
	F1b	F2b	10	10	10	9
Number of males failing to induce pregnancy	F0	F1a	7	6	2	7
	F1b	F2a	6	10	8	9
	F0	F1b	9	8	4	**16**
	F1b	F2b	13	20	15	17
Number of females failing to become pregnant	F0	F1a	5	4	2	5
	F1b	F2a	5	9	7	8
	F0	F1b	7	6	4	**14**
	F1b	F2b	11	19	11	14
Number of pregnant females	F0	F1a	25	26	28	25
	F1b	F2a	27	23	25	23
	F0	F1b	21	24	26	**16**
	F1b	F2b	21	13	21	17
Number of females with litters at term	F0	F1a	25	26	28	25
	F1b	F2a	27	23	25	23
	F0	F1b	21	23	26	**16**
	F1b	F2b	21	13	21	17

* Some values in the addendum from Nov. 2007 were wrong and have been changed in this Table. This has no consequences for the conclusions.

The second mating of the F0 and F1b parents is shaded and the high dose values for the F0/F1b mating are emboldened.

Whilst the reproductive data for the high dose F0/F1b mating may appear to be inconsistent within that mating, they are consistent with those for the F1b/F2b mating (high dose and controls) where there is no treatment related effect. Therefore, the differences noted for the F0/F1b mating are considered not to indicate an effect of abamectin since this was not reproduced in the definitive second mating of the F1b parents. There are also no effects of treatment for the first matings of both the F0 and F1b parents. The lack of reproducibility of the findings is considered to provide strong evidence that abamectin does not have an adverse effect on mating performance and the number of matings. No adverse effects on reproductive performance were reported for the closely related human and veterinary anti-parasitic drug, ivermectin (WHO JECFA, 1991).

Characteristics

Reference/notifier	:	[redacted] (1984e)	Exposure	:	F0: 68 days prior to mating until 50 days after weaning of F1b litter; (P: 259 days (m) and 177 days (f)) F1b: from weaning until approximately 30 days after weaning of F2b (273 days)
Type of study	:	Two-generation study in rats with abamectin technical.	Doses[1]	:	0, 0.05, 0.12 and 0.4 mg/kg bw/day
Year of execution	:	1982-1983	Vehicle	:	sesame oil
Test substances	:	abamectin technical (MK-0936, purity [redacted])	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 416
Species	:	Rats (Crl:COBS CD (SD) BR	Acceptability	:	Acceptable
Group size	:	30/sex/dose	$NOAEL_{parent}$	:	≥ 0.4 mg/kg bw/ day
			$NOAEL_{pup}$	:	0.12 mg/kg bw/ day
			$NOAEL_{reproduction}$	:	≥ 0.4 mg/kg bw/ day

1: dose levels were selected on the basis of results from reproduction studies and a multigeneration range-finding study in the rat (not submitted)

Study design

The study was performed in accordance with OECD guideline 416, with the folllowing deviations: food and water consumption were not determined, reproductive organs of animals failing to mate were not examined microscopically, testes and epididymides were weighed together, P generation females were not subjected to necropsy and only 10 male and 25 female F1 parental animals were subjected to necropsy.

F0 rats were mated twice in order to produce two litters, F1a and F1b. P generation females not producing a litter were killed and discarded after the reproductive status had been confirmed. All other P generation females were killed and discarded after weaning of the F1b litters. P generation males were killed, testes and epididymides weighed (together) and then discarded. F1a litters were weaned, killed and discarded without further examination on day 21 post partum. From the F1b litters, 32 pups/sex/group (2 pups/sex/litter) were selected and mated twice to produce F2a and F2b generations in a similar manner to

the P generation. All other F1b progeny were killed and discarded. Five F2b pups/sex/group were selected for full gross examination, organ weights and microscopic pathology evaluation, and 10 pups/sex/group were killed for skeletal examination. All other F2b pups were killed at weaning (eyes preserved). Thirty days after weaning of the last F2b litter, 10 male and 25 female F1b parental animals/group were selected for necropsy, organ weights and histopathology. The remaining F1b parental animals were killed and discarded (testes and epididymides were weighed and preserved, eyes were preserved).

Results

Results of the study are summarized in table below.

Results of two-generation study in rats with abamectin technical.

Dose (mg/kg bw/day)		0		0.05		0.12		0.4		dr
		m	f	m	f	m	f	m	f	
F0 animals										
	Mortality	No treatment-related deaths								
	Clinical signs	No toxicologically relevant effects								
	Body weight gain -during lactation F1a -during lactation F1b					i 	is d d	is 	is ds d	mf f
	Food consumption	Not performed								
	Water consumption	Not performed								
	Sperm parameters	Not performed								
	Testes and epididymides weight	No toxicologically relevant effects								
	Pathology	Not performed								
	macroscopy									
	microscopy									
Effect during F1a mating		No toxicologically relevant effects (see tables above)								
Effects during F1b mating		No toxicologically relevant effects (see tables above)								
F1 pups										
Effects on F1a litters	-pup mortality (%), days 5-15 -pup weight (days 7-21) -incidence of total litter loss (%) -lactation index [a](% survival day 4-21)	6.3 8.0 99.5		2.7 3.8 100		3.0 0.0 99.2		42.2 (is) ds 28.0 (is) 52.7 (ds)		

		0	0.05	0.12	0.4	dr
Effects on F1b litters	-pup mortality (%), days 5-15	2	2	4	33 (is)	
	-pup weight (days 7-21)				ds	
	-incidence of total litter loss (%)	0.0	0.0	0.0	25.0 (is)	
	-lactation index [a] (% survival day 4-21)	98.0	98.5	99.2	60.0 (ds)	
	Clinical signs F1a and F1b -thin and not nursing				i	
	Sex ratio	No toxicologically relevant effects				
	Skeletal evaluation	No toxicologically relevant effects				
	Retinal anomaly				i	
F1 animals						
	Mortality	No treatment-related deaths				
	Clinical signs	No toxicologically relevant effects				
	Body weight			ds	ds ds	
	Food consumption	Not performed				
	Water consumption	Not performed				
	Oestrus cycle	Not performed				
	Sperm parameters	Not performed				
	Organ weights	No toxicologically relevant effects				
	Pathology					
	macroscopy					
F2a pups	Pup mortality (%)	1.8	1.3	1.2	6.7 (is)	
	Body weight/litter, day 7-21				ds	
	Male pups/litter, day 1 (%)	58	55	52	46	dr
	Lactation index				ds	
	Viability index (day 4-14)				ds	
	Gross litter observation -thin -weak -not nursing				i i i	
F2b pups	Pup mortality (%)	4.2	1.6	1.5	8.6	
	Body weight/litter, day 7-21				ds	
	Male pups/litter, day 1 (%)	58	50	53	46	
	Lactation index				ds	
	Viability index (day 4-14)				ds	
	Gross litter observation -thin -weak -not nursing				i i i	
	pathology microscopy - retinal anomaly				is is	

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

a: lactation index = percentage of pups surviving postculling to day 21 of lactation.

Average body weight gain was higher in the mid and high dosed F0 animals compared to controls.

However, during the F0-F1a and F0-F1b lactation periods, average body weight gain was (significantly) lower in the 0.12 and 0.40 mg/kg bw/day dams compared to controls. Since this effect was relatively small, and the females did gain weight, although to a lesser extent than controls, this effect is considered not relevant for setting the NOAEL.

Pup mortality for both F1a and F1b litters was significantly increased in the high dose group, with most pups dying days 5-15 postpartum. Post mortem examination of F1b weanlings showed retinal anomalies (single or multiple retinal folds of many layers of the retina) in 3 out of 4 males in the highest dose group.

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

In both F2a and F2b litters treated at 0.4 mg/kg bw/day pup mortality significantly increased during the course of lactation, and the associated viability and lactation indices significantly decreased. Pup weights in the high dose group was unaffected by treatment directly after birth and for the first few days, but were significantly reduced from day 7 to day 21. This was associated with increased numbers of pups that were thin, weak and not nursing. The number of male pups was decreased in the high dose group (F2a and F2b).

Post mortem examination of F2b weanlings showed retinal anomalies, with characteristics identical to those observed in F1b animals in 10/63 males and 18/66 females in the highest dose group. As in the F1 pups, it is considered that these retinal anomalies are transient and confined to the pup stage.

Acceptability

The study is considered acceptable.

Conclusions

It is concluded that in the multigeneration study in the rat the NOAEL for parental and reproduction toxicity is 0.4 mg/kg bw/day, i.e. the highest dose tested.

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

Based on the additional information it is concluded that a classification for fertility effects is not necessary.

Reliability Indicator	1	
Data Protection Claim	Yes	

In DAR: **STUDY 9** (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)

Characteristics

Reference/notifier	:	[redacted] (1988b)	Exposure	:	F: 15 days prior to mating, throughout cohabitation and gestation and throughout lactation until day 20
Type of study	:	One generation reproductive toxicity study in the rat.	Doses	:	0, 0.06, 0.12 and 0.40 mg/kg bw/day
Year of execution	:	1987	Vehicle	:	sesame oil
Test substances	:	8,9-Z isomer of avermectin B1a (purity [redacted])	GLP statement	:	yes
Route	:	Oral (gavage)	Guideline	:	OECD 415
Species	:	Sprague-Dawley rats (Crl:CD[SD])BR strain	Acceptability	:	Acceptable as additional study
Group size	:	20 females/dose	NOAEL $_{maternal}$	:	0.4 mg/kg bw/ day
			NOAEL $_{pup}$	:	0.12 mg/kg bw/ day

1: day 0 of gestation= day sperm were observed in the vagina

Study design

According to OECD guideline 415, with the following deviations: males not treated, F1 progeny not weighed on day 4, dead and culled pups were not subjected to gross necropsy and maternal reproductive organs were not subjected to histopathological examination.

Four groups of 20 female Sprague-Dawley rats were treated orally, by gavage, for 15 days prior to mating, throughout cohabitation and gestation, and through lactation until day 20 with the 8,9-Z isomer of avermectin B1a as a solution in sesame oil, at dose levels of 0 (vehicle only), 0.06, 0.12 and 0.40 mg/kg bw/day. All maternal animals were observed daily for clinical signs. Maternal body weights were recorded weekly during the pre-mating and cohabitation periods, on days 1, 6, 12, 16, 18 and 20 of gestation and daily thereafter until parturition, and on days 0, 7, 14 and 20 of lactation. Maternal food consumption was measured at 5-day intervals twice during pre-mating, 3 times during gestation and twice during lactation. After 15 days treatment, the females were paired 1:1 with untreated males of the same strain for a maximum of 14 days. The females were allowed to litter naturally. Neonatal pups were examined externally on the day of birth. Dead pups were given a visceral examination limited to trachea and

oesophagus only. The litters were culled to 8 pups/litter on day 3 of lactation by random selection but with a balanced sex ratio where possible. Excess pups were killed and discarded without further examination. Pup weight and sex were recorded on days 0, 7, 14 and 21 of lactation and clinical signs were recorded daily until sacrifice on day 21 *post partum*.

Post mortem examination of F1 progeny was limited to the eyes, which were preserved and examined histologically. Complete retinal cross-sections of both eyes were examined.

Females of the P generation not giving birth were killed and subjected to necropsy on day 24 of presumed gestation. All other maternal animals were killed and subjected to gross necropsy and metrial gland count on days 21 - 23 *post partum*.

Results

No deaths or treatment-related clinical signs occurred at any dose level during the course of the study. There were no treatment-related effects on maternal weight gain or food consumption and no treatment-related gross findings at necropsy at any dose level. There were no treatment-related effects at any dose level on mating performance as assessed by mean day of mating, numbers of matings and pregnancies and duration of gestation. One pup from the 0.12 mg/kg bw/day group had anencephaly and ethmocephaly at birth and one pup from the 0.06 mg/kg bw/day group, with a domed head, was found to be severely hydrocephalic at necropsy on day 21. There were no other external malformations and pup growth was unaffected. In the highest dose group, the sex ratio (m:f) and post-natal death were increased (see table). Although post-implantation survival was significantly reduced ($p < 0.05$) in the 0.12 mg/kg bw/day group (84.9%) compared with the control value of 94.6%, the difference was due largely to one female bearing one dead pup at birth. Since there was no dose-relationship, the difference is considered to be incidental to treatment. Gross and histomorphological examination of the eyes of F1 generation progeny revealed no treatment-related effects.

Dose (mg/kg bw/day)	0	0.06	0.12	0.40
No. pregnant / no. mated	18 / 20	18 / 20	18 / 20	18 / 20
% post-implantation survival (litter mean)	94.6	94.1	84.9*	94.2
Sex ratio at birth (M:F)	1 : 0.79	1 : 0.77	1 : 0.91	1 : 0.98
No. (%) post-natal deaths	10 (3.94)	6 (2.48)	10 (4.59)	16 (6.53)

* $p < 0.05$

Acceptability

The study is acceptable as investigative study.

Conclusions

A NOAEL for maternal toxicity and reproductive effects in rats for the 8,9-Z isomer of avermectin B1a in this study is established as 0.4 mg/kg bw/day, based on no effects on female fertility and reproductive performance at the highest dose level tested. The $NOAEL_{fetal}$ is established as 0.12 mg/kg bw/day based on an increase in post-natal deaths and in sex-ratio.

98/8 Doc IIIA section No.	6.8.2/ 02	Reproductive Toxicity – Two Generations	Official use only
91/414 Annex Point addressed	II 5.8.1/ 03	Two Generation reproductive toxicity	

Title:	8,9-isomer of avermectin B1 oral maternotoxicity study in mice	
Lab Report Number:	No. TT 84-722-0	
Authors:	[redacted]	
Test Substance:	8,9-Z isomer of 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 1 (6.8 Further toxicological studies/6.8.1 Toxicity studies of metabolites/6.8.1.2 Reproductive toxicity)

Characteristics

Reference/notifier	:	[redacted] (1986d)	Exposure	:	Day 6-15 of gestation[1]
Type of study	:	Oral maternotoxicity study in mice with the 8,9-isomer of avermectin B1a.	Doses	:	0, 1.5, 3.0, 6.25, 12.5, 25.0 and 50.0 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 (gastric intubation)	Guideline	:	unknown
Species	:	Mice (Crl:CF-1 (BR))	Acceptability	:	Acceptable as investigative study
Group size	:	7-13 mated females/dose	NOAEL maternal	:	< 1.5 mg/kg bw/ day
			NOAEL fetal	:	< 1.5 mg/kg bw/ day

1: day 0 = day of vaginal plug observed

Study design

Seven groups of 7-13 mated female mice (Crl:CF-1 (BR)) were treated orally (gavage) with the 8,9-Z isomer of avermectin B1a in sesame oil at dose levels of 0, 1.5, 3.0, 6.25, 12.5, 25.0 and 50.0 mg/kg bw/day from day 6 to day 15 of gestation. Treatment at dose levels of 3.0 mg/kg bw and higher was discontinued after a single dose due to mortality. Clinical signs were recorded at least once a day. 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-isomer of avermectin B1a.

Dose (mg/kg bw/day)	0	1.5	3.0	6.25	12.5	25.0	50.0
Mean weight gain day 6 - 17 (g)	19.5	17.7	-	-	-	-	-
Overall pregnancy incidence[a]	13 / 13	8 / 11	11 / 11	7 / 7	7 / 8	7 / 8	8 / 8
Live pregnant	13	7	*	*	*	*	*
Live not pregnant	0	3	*	*	*	*	*
Dead pregnant	0	1	*	*	*	*	*
Mean no. implants/female	14.1	12.6	*	*	*	*	*
No. (mean/female) resorptions	19 (1.46)	5 (0.71)	*	*	*	*	*
No. (mean/female) dead foetuses	1 (0.08)	0 (0.00)	*	*	*	*	*
Live foetuses (mean/female)	12.5	11.9	*	*	*	*	*
Sex ratio (M : F)	1 : 0.94	1 : 0.77	*	*	*	*	*
Mean live fetal weight (g)	0.89	0.85	*	*	*	*	*

* females from dose groups 3.0 mg/kg bw/day and higher were sacrificed on days 6 - 8

a: initially, 12 females were assigned to each of the groups. Due to deaths before all animals had been given at least one dose, the remaining undosed animals were reassigned to two new groups dosed 1.5 and 3.0 mg/kg bw/day.

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

Dose (mg/kg bw/day)	0	1.5
No. foetuses (litters) examined	163 (13)	83 (7)
No. foetuses (litters) with malformations	0 (0)	25 (4)
% foetuses with malformations	0	30.1
No. foetuses (litters) with cleft palate	0 (0)	24 (4)
No. with exencephaly + omphalocele	0 (0)	1 (1)

After one dose, there were 2 deaths each in the 3, 12.5, 25 and 50 mg/kg bw/day groups and 3 deaths in the 6.25 mg/kg bw/day group. These groups were terminated on days 6-8 of gestation. There was one death in

the 1.5 mg/kg bw/day group after 2 doses (day 8 of gestation). Some of the dead females were comatose and/or moribund a few hours prior to death. The overall weight gain during the treatment period at 1.5 mg/kg bw/day was 9% lower than the controls. No gross lesions were observed at necropsy in maternal animals of the 1.5 mg/kg bw/day group. In this lowest dose group, an increased incidence (24 fetusus from 4 litters) of cleft palate was observed compared to controls (none). One fetus from a litter in which another fetus had cleft palate, had encephaly and omphalocele. The overall external malformaltion incidence in the 1.5 mg/kg bw/day group was 30% compared with a control incidence of 0%. The sex ratio (m:f) was lower in the treated group (1:0.77) compared to the control group (1:0.94). 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.

Acceptability

The study is acceptable as investigative study.

Conclusions

Groups dosed at 3.0 mg/kg bw/day and above were terminated due to increased mortality after one dose. Maternotoxicity and teratogenicity were observed at the lowest dosage tested, 1.5 mg/kg bw/day, therefore a NOAEL in this study could not be established, and the LOAELs for both maternotoxicity and teratogenicity in this study are 1.5 mg/kg bw/day.

SYNGENTA CONCLUSION

Conclusion:	The 8,9-Z isomer of avermectin B_{1a} elicits both maternal toxicity and a teratogenic response at 1.5 mg/kg bw/day. No-observed-effect-levels (NOEL) for maternal and foetal toxicity could not be established since a treatment-related maternal death occurred and an excess incidence of cleft palates occurred at the lowest dose level employed.	

Reliability Indicator	1	
Data Protection Claim	Yes	

Overall summary of the developmental studies

Many developmental studies have been performed with abamectin, avermectin B1a and the 8,9-Z isomer. During the evaluation of abamectin for Plant Protection Products, the RMS was requested to provide a comparative table with detailed results of the different developmental studies (for abamectin, avermectin B1a and 8,9-Z isomer) to be discussed by the experts with regard to classification. This table has been presented in the addendum (Febr. 2008) and is also presented below.
Furthermore, the notifier sent important historical background data and these are presented under the table.

Copy from the revised addendum (Febr. 2008):
The principal findings, NOAELs and LOAELs in the developmental toxicity studies on abamectin, avermectin B1a and the 8,9-Z isomer of avermectin B1a are shown in the table below. Where relevant, the most critical effects (for classification and labelling) are included in the form of a small table. Furthermore, some relevant historical control data which have been submitted by the notifier are presented after the table. For the discussion on classification and labelling, in principle also the effects on the pups in the 2-generation study in rats should be taken into account. However, as explained in more detail at B.6.10.3 (in the CAR this is explained in "10. Mechanistic data"), the neonatal rat is sensitive to abamectin toxicity because the placental barrier and blood-brain barrier is incompletely formed at birth, which is considered in contrast to man. Since such sensitive period with limited P-glycoprotein expresion is not present in man, the effects observed in neonatal rats are considered not relevant for human risk evaluation and therefore also not for classification and labelling.

It should be noted that developmental toxicity studies on the 8,9-Z isomer of avermectin B1a are inappropriate to include in the discussion of the hazard classification of abamectin since the isomer is a photodegradate and is not a component of the active substance.

Developmental toxicity studies were conducted on abamectin, avermectin B1a and its principal photodegradate on plants (the 8,9-Z isomer of avermectin B1a). Many studies were performed with the CF-1 mouse, which is very sensitive to the observed developmental effects. Based on a recent extensive overview of the literature, it is however concluded that the CF-1 mouse is not relevant for human risk assessment (see B.6.8.2 in this addendum; in the CAR this is explained in "10. Mechanistic data") and the results of studies with the CF-1 mouse are therefore also not relevant for classification and labelling.
The studies with the CF-1 mouse are therefore marked grey in the table below.

In PRAPeR 39 (Dec. 2007) it was decided to propose the classification:
Category 3, R63 (R61?).

<table>
<tr><th>Test substance / species / study type</th><th>NOAEL mg/kg bw/day</th><th>LOAEL mg/kg bw/day</th><th>Critical effects</th><th>Reference</th></tr>
<tr><td colspan="5">Abamectin technical</td></tr>
<tr><td>Abamectin technical (vehicle sesame oil) / rats (CRCD) / Oral developmental study</td><td>Maternal: 1.6

Developm: 0.8</td><td>>1.6

1.6</td><td><u>Maternal</u>: no effects observed at highest dose (1.6 mg/kg bw/day)
<u>Developmental</u>: Cleft palate, lumbar rib and lumbar rib count variation</td><td>[redacted] (1982a)</td></tr>
<tr><td colspan="5">
<table>
<tr><th>Dose (mg/kg bw/day)</th><th></th><th>0</th><th>0.4</th><th>0.8</th><th>1.6</th></tr>
<tr><td></td><td>No. of dead fetuses/no. of fetuses studied</td><td>0/319</td><td>0/320</td><td>0/279</td><td>0/326</td></tr>
<tr><td></td><td>Malformations
-exencephaly
-cleft palate</td><td></td><td></td><td>
1[b]</td><td>1[a]
1</td></tr>
<tr><td></td><td>Skeletal deviations
-lumbar rib (no / %)
-lumbar count variation (no / %)
- no. of litters with fetal variations / no. of litters examined</td><td>
44 / 14
1 / 0.3

13 / 23</td><td>
41 / 13
1 / 0.3

18 / 24</td><td>
45 / 16
1 / 0.4

14 / 24</td><td>
72 / 22
5 / 1.5

16 / 24</td></tr>
</table>
a: conjoined twin

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

See also the historical control data below.
The historic control data show that a single incidence of cleft plate occurred in studies conducted in the testing facility over the 5-year time period appropriate to this study. The single incidence of exencephaly at 1.6 mg/kg bw/day was in association with a conjoined twin, which is a spontaneous congenital abnormality. There were no incidences of exencephaly/conjoined twin in the historic control data. The increased incidences of extra (lumbar) rib and vertebral (lumbar) count variation at 1.6 mg/kg bw/day were within the historic control range. The historic control data show that the incidences of both findings are very variable in control fetuses.
</td></tr>
<tr><td>Abamectin technical (vehicle sesame oil) / rabbits (New Zealand albino) / Oral developmental study</td><td>Maternal: 1.0

Developm: 1.0</td><td>2.0

2.0</td><td><u>Maternal</u>: decreased water and food consumption and weight loss during gestation, increased number of resorptions.
<u>Developmental</u>: cleft palate, omphaloceles, clubbed fore-feet and delayed ossification</td><td>[redacted] (1982b)</td></tr>
</table>

Dose (mg/kg bw/day)		0	0.5	1.0	2.0
	No. of dead fetuses/no. of fetuses studied	0/97	1/91	5/100	0/121
	% malformed fetuses	3.1	4.4	4.0	12.4
	External observations and visceral deviations				
	-cleft palate	0	0	0	2[a]
	-clubbed fore-foot	1	0	2	5[a]
	-omphaloceles	1	0	0	2[a]
	Skeletal deviations				
	-sternebral malformation	0	0	0	3
	-incompletely ossified sternebra	17	17	16	42
	-incompletely ossified metacarpal	8	15	7	33
	-incompletely ossified phalanx	19	27	12	31

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

Avermectin B1a

Avermectin B1a / CF-1 mice / Oral developmental study	Maternal: <0.1 Developm: 0.2	0.1 0.4	Maternal: mortality and clinical signs Developmental: Cleft palate	[redacted] (1976)

Dose (mg/kg bw/day)		0	0	0.1	0.2	0.4	0.8
Fetus examination	No. dead/no. studied	2/292	2/270	1/261	0/227	2/244	0/199
	% malformed fetuses	0.34	0.37	1.1	0.44	2.0	5.0
External foetal examination	Malformations						
	- exencephaly	0	0	2	1	0	0
	- ablepharia	0	0	2	1	0	0
	- micrognathia	0	0	1	0	0	0
	- cleft palate	0	0	0	0	5	10
	- protruding tongue	1	1	0	0	0	0
	- aglossia	0	0	1	0	0	0

Avermectin B1a / CF-1 mice / Oral developmental	Maternal: 0.2 Developm: 0.2	0.4 0.4	Maternal: mortality and clinical signs Developmental: Cleft palate	[redacted] (1977)

Dose (mg/kg bw/day)		**0**	**0**	**0.1**	**0.2**	**0.4**	**0.8**
Fetus examin-ation	No. dead/no. studied	0/184	0/234	0/195	1/242	0/165	1/199
	% malformed fetuses	1.1	0.85	1.5	0	2.4	5.0
External foetal examin-ation	Malformations						
	- cleft palate	0	1	1	0	4	5
	- exencephaly	1	0	0	0	0	3
	- preaxial polydactyly	0	0	1	0	0	0
	- ablepharia	1	1	0	0	0	3
	- reduced ear flap	1	0	0	0	0	0
	- corkscrew tail	0	0	1	0	0	0
	- outward rotation of hind leg	0	0	1	0	0	0

8,9-Z isomer of avermectin B1a

8,9-Z isomer / CF-1 strain mice / Oral maternal toxicity study	Maternal: < 1.5 Fetal: < 1.5	Maternal: 1.5 Fetal: 1.5	Maternal: death. Developmental: excess incidences of cleft palate and omphalocele	[redacted] (1986d)
8,9-Z isomer / CF-1 strain mice / Oral maternal toxicity study	Maternal: 0.1 Fetal: 0.05	Maternal: 0.5 Fetal: 0.1	Maternal: death. Developmental: excess incidences of cleft palate and exencephaly	[redacted] (1986e)
8,9-Z isomer / CF-1 strain mice / Oral developmental toxicity study	Maternal: 0.06 Fetal: 0.015	Maternal: > 0.06 Fetal: 0.03	Maternal: no effects Developmental: exencephaly and incomplete ossification	[redacted] (1986f)
8,9-Z isomer / CF-1 strain mice / Oral developmental toxicity study	Maternal: ~~0.015~~ 0.1 Fetal: ~~0.015~~ 0.03	Maternal: ~~0.03~~ 0.5 Fetal: ~~0.03~~ 0.1	Maternal: mortality ~~resorptions~~ Developmental: cleft palate ~~and exencepaly~~	[redacted] (1986g)

8,9-Z isomer / Sub-populations of abamectin-sensitive and insensitive CF-1 strain mice / Oral developmental toxicity study	$Maternal_{insensit.}$:1.5 $Maternal_{sensitive}$: <0.2-1.0 Fetal : could not be established (only one litter)	Insensitive: > 1.50 Sensitive: 0.2-1.0 Fetal: could not be established (only one litter)	$\underline{Maternal}_{insensitive}$: no effects $\underline{Maternal}_{sensitive}$: decreased bw gain during day 6-16 of gestation (Developmental: (cleft palate))	[redacted] (1996a)
8,9-Z isomer / CF-1 mice of known P-glycoprotein genotype / Exploratory oral developmental toxicity study	Maternal: <1.50 Developmental: < 1.50	1.50 1.50	Maternal: decreased weight gain during day 6-18 of gestation Developmental: excess incidence of cleft palate in sensitive fetuses.	[redacted] (1996a)
8,9-Z isomer / CD-1 strain mice / Oral developmental toxicity study	Maternal: 3.0 Fetal: < 0.75	Maternal: > 3.0 fetal : 0.75	Maternal: no effects Developmental: cleft palate	[redacted] (1996b)

Dose (mg/kg bw/day)	0	0.75	1.5	3.0
Cleft palate (fetal incidence (%) / litter incidences (% of litters))	0 / 0 (0)	2 (0.8) / 2 (9.5)	1 (0.4) / 1 (5)	4 (1.5) / 3 (13.6)
Fetuses with malformation (%)	0	5	3	6
Litters with malformation (%)	0	24	10	23
Fetuses with variation (%)	2	2	4	12
Litters with variation (%)	10	5	10	18

See also the historical control data below.

8,9-Z isomer / CD strain mice / Oral developmental toxicity study	Maternal: 1.0 Fetal: 1.0	Maternal: > 1.0 Fetal: > 1.0	Maternal: no effects. Developmental: no effects.	[redacted] (1988b)

Historical control data CR CD rat

Cleft palate

The historic control data show that a single incidence of cleft plate occurred in studies conducted in the testing facility over the 5-year time period appropriate to this study (Table 1).

TABLE 1: Historical control incidence of cleft palate in oral maternotoxicity/teratogenicity studies in CD rat

Dose mg/kg/day	Report TT #	Litters examined	Litters with cleft palate		Fetuses examined	Fetuses with cleft palate	
		N	N	%	N	N	%
Control	79-7090	25	0	0	332	0	0
	79-7150	46	0	0	636	0	0
	79-7160	19	0	0	246	0	0
	79-7210	17	0	0	234	0	0
	80-7010	20	0	0	279	0	0
	80-7020	24	1	4.2	328	1	0.30
	80-7080	25	0	0	308	0	0
	80-7120	22	0	0	293	0	0
	80-7150	25	0	0	321	0	0
	80-7170	24	0	0	308	0	0
	81-7010	24	0	0	307	0	0
	81-7020	24	0	0	326	0	0
	81-7110	24	0	0	330	0	0
	81-7130	25	0	0	321	0	0
	81-7180	25	0	0	324	0	0
	82-7050	23	0	0	319	0	0
	83-7030	23	0	0	328	0	0
	83-7100	23	0	0	317	0	0
	83-7101	17	0	0	210	0	0

	83-7160	22	0	0	305	0	0
	84-7010	24	0	0	321	0	0
	84-7060	19	0	0	231	0	0
	84-7140	22	0	0	324	0	0
Historic control	From 23 studies between 1979 to 1984		Mean	0.18		Mean	0.02
			Minimum	0		Minimum	0
			Maximum	4.2		Maximum	0.30

Exencephaly

The single incidence of exencephaly at 1.6 mg/kg bw/day was in association with a conjoined twin, which is a spontaneous congenital abnormality. There were no incidences of exencephaly/conjoined twin in the historic control data.

Extra (lumbar) rib and vertebral (lumbar) count variation

The increased incidences of extra (lumbar) rib and vertebral (lumbar) count variation at 1.6 mg/kg bw/day were within the historic control range (Tables 2 and 3). The historic control data show that the incidences of both findings are very variable in control fetuses.

TABLE 2. Historical control incidence of extra rib (lumbar) in oral maternotoxicity/teratogenicity studies in CD rat

Dose mg/kg/day	**Report TT #**	**Litters examined**	**Litters with extra rib**		**Fetuses examined**	**Fetuses with extra rib**	
		N	**N**	**%**	**N**	**N**	**%**
	79-7090	25	21	84.0	332	94	28.3
	79-7150	46	39	84.8	636	177	27.8
	79-7160	19	12	63.2	246	45	18.3
	79-7210	17	11	64.7	234	34	14.5
	80-7010	20	15	75.0	279	75	26.9
	80-7020	24	14	58.3	328	49	14.9
	80-7080	25	18	72.0	308	79	25.6
	80-7120	22	14	63.6	293	55	18.8
	80-7150	25	13	52.0	321	30	9.3
	80-7170	24	13	54.2	308	35	11.4

	81-7010	24	8	33.3	307	21	6.8
	81-7020	24	16	66.7	326	30	9.2
	81-7110	24	12	50.0	330	37	11.2
	81-7130	25	17	68.0	321	45	14.0
	81-7180	25	14	56.0	324	37	11.4
	82-7050	23	13	56.5	319	44	13.8
	83-7030	23	14	60.9	328	41	12.5
	83-7100	23	16	69.6	317	62	19.6
	83-7101	17	6	35.3	210	12	5.7
	83-7160	22	12	54.5	305	43	14.1
	84-7010	24	7	29.2	321	12	3.7
	84-7070	19	5	26.3	231	10	4.3
	84-7140	22	14	63.6	324	62	19.1
Historic control	From 23 studies between 1979 to 1984		Mean Minimum Maximum	58.3 26.3 84.8		Mean Minimum Maximum	14.8 3.7 28.3

TABLE 3. Historical control incidence of vertebral count variation in oral maternotoxicity/teratogenicity studies in CD rat

Dose mg/kg/day	**Report TT #**	**Litters examined**	**Litters with vertebral count variation**		**Fetuses examined**	**Fetuses with vertebral count variation**	
		N	**N**	**%**	**N**	**N**	**%**
Control	79-7090	25	0	0	332	0	0
	79-7150	46	5	10.9	636	6	0.9
	79-7160	19	1	5.3	246	1	0.4
	79-7210	17	1	5.9	234	1	0.4
	80-7010	20	1	5.0	279	1	0.4
	80-7020	24	3	12.5	328	3	0.9
	80-7080	25	7	28.0	308	9	2.9
	80-7120	22	1	4.5	293	1	0.3
	80-7150	25	0	0	321	0	0
	80-7170	24	0	0	308	0	0
	81-7010	24	1	4.2	307	1	0.3
	81-7020	24	0	0	326	0	0

	81-7110	24	1	4.2	330	1	0.3
	81-7130	25	1	4.0	321	1	0.3
	81-7180	25	1	4.0	324	2	0.6
	82-7050	23	1	4.3	319	1	0.3
	83-7030	23	0	0	328	0	0
	83-7100	23	0	0	317	0	0
	83-7101	17	0	0	210	0	0
	83-7160	22	1	4.5	305	1	0.3
	84-7010	24	0	0	321	0	0
	84-7060	19	1	5.3	231	1	0.4
	84-7140	22	1	4.5	324	2	0.6
Historic control	From 23 studies between 1979 to 1984		Mean Minimum Maximum	4.7 0 28.0		Mean Minimum Maximum	0.4 0 2.9

Historical control data CD-1 strain mice

The historical control data below are copied from the study report of [redacted] (1996b), together with additional data provided by the registrant. The historical control database of developmental toxicity parameters for CD-1 mice consists of seven studies performed during 1995 – 1998 in the same laboratory as the study performed by [redacted].

Historical control incidence of cleft palate in oral maternotoxicity/teratogenicity studies in CD-1 mice

Dose (mg/kg/gday)	**Study No.**	**Number examined**		**Cleft Palate**			
		Litters	**Foetuses**	**No. of litters affected**	**% litters incidence**	**No. of foetuses affected**	**% foetuses incidence**
Control	957130	23	291	0	0	0	0
	957135	21	267	0	0	0	0
	957136	24	301	3	12.5	4	1.3
	957365	10	101	1	10.0	2	2.0
	967020	24	297	2	8.3	4	1.3
	967320*	**20**	**231**	**0**	**0**	**0**	**0**

987060	15	208	0	0	0	0
987250	10	131	1	10.0	1	0.8

* 8,9-Z isomer study

	Evaluation by Competent Authorities
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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*12 november 2007; updated January 2009*
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[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	

Summary from abamectin revised addendum (Febr. 2008). This is included for

information.

Based on a recent extensive overview of the literature, it is concluded that the CF-1 mouse is not relevant for human risk assessment (see B.6.8.2 in this addendum; in the CAR this is explained in "10. Mechanistic data"). The studies with the CF-1 mouse are therefore marked grey in the table below.

Reproduction and teratogenicity studies with abamectin technical

Test substance	Duration, route	Species	NOAEL (mg/kg bw/day	LOAEL (mg/kg bw/day)	Critical effects	Reference/ Notifier
Abamectin technical (vehicle sesame oil)	Oral 2-generation study	rat	Parental: 0.4 Offspring: 0.12 Reproduction: 0.4	Parental: >0.4 Offspring: 0.4 Reproduction: >0.4	Parent: increased mating time, decreased number of males and females mating, increased duration of cohabitation, increased number of dams with prolonged interestrus, less females littering Fetes/pups: increased pup mortality, retarded weight gain pups (F1 and F2), increased incidence of total litter loss, decreased lactation index, increased incidence of retinal anomaly in the eyes of pups (F1 and F2)	[redacted] (1984e)
Abamectin technical (vehicle sesame oil)	Oral developmental study	rat	Maternal: 1.6 Developm: 0.8	>1.6 1.6	Cleft palate, lumbar rib and lumbar count variation	[redacted] (1982a)
Abamectin technical (vehicle sesame oil)	Oral developmental study	rabbit	Maternal: 1.0 Developm: 1.0	2.0 2.0	Maternal: decreased water and food consumption and weight loss during gestation, increased number of resorptions. Developmental: cleft palate, omphaloceles, clubbed fore-feet and delayed ossification	[redacted]. (1982b)

Summary of studies on metabolites

Study/species dose levels	Test article	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	Major effects	Reference
Oral maternal toxicity study; CF-1 strain mice	8,9-Z isomer	Maternal: < 1.5 Fetal: < 1.5	Maternal: < 1.5 Fetal: 1.5	Death. Excess incidences of cleft palate and omphalocele.	[redacted] (1986d)

Study/species dose levels	Test article	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	Major effects	Reference
Oral maternal toxicity study; CF-1 strain mice	8,9-Z isomer	Maternal: 0.1 Fetal: 0.05	Maternal: 0.5 Fetal: 0.1	Death. Excess incidences of cleft palate and exencephaly.	[redacted] (1986e)
Oral developmental toxicity study; CF-1 strain mice	8,9-Z isomer	Maternal: 0.06 Fetal: 0.015	Maternal: > 0.06 Fetal: 0.03	No effects Exencephaly and incomplete ossification	[redacted] (1986f)
Oral developmental toxicity study; CF-1 strain mice	8,9-Z isomer	Maternal: ~~0.015~~ 0.1 Fetal: ~~0.015~~ 0.03	Maternal: ~~0.03~~ 0.5 Fetal: ~~0.03~~ 0.1	~~mortality resorptions~~ cleft palate ~~and exencepaly~~	[redacted] (1986g)
Oral developmental toxicity study; Sub-populations of abamectin-sensitive and insensitive CF-1 strain mice	8,9-Z isomer	$Maternal_{insensitive}$: 1.5 $Maternal_{sensitive}$: <0.2-1.0 Fetal : could not be established (only one litter)	Insensitive: > 1.50 Sensitive: 0.2-1.0 Fetal: could not be established (only one litter)	No effects. Decreased bw gain during day 6-16 of gestation - (cleft palate)	[redacted] (1996a)
Exploratory oral developmental toxicity study; CF-1 mice of known P-glycoprotein genotype	8,9-Z isomer	Maternal: <1.50 Developmental: < 1.50	1.50 1.50	Decreased weight gain during day 6-18 of gestation Excess incidence of cleft palate in sensitive fetuses.	[redacted] (1996a)

Study/species dose levels	Test article	NOAEL (mg/kg bw)	LOAEL (mg/kg bw)	Major effects	Reference
Oral developmental toxicity study; CD-1 strain mice	8,9-Z isomer	Maternal: 3.0 Fetal: < 0.75	Maternal: > 3.0 fetal : 0.75	No effects Cleft palate	[redacted] (1996b)
Oral developmental toxicity study; CD strain rats	8,9-Z isomer	Maternal: 1.0 Fetal: 1.0	Maternal: > 1.0 Fetal: > 1.0	No effects. No effects.	[redacted] (1988a)
Oral one-generation female reproduction study; CD strain rat	8,9-Z isomer	Maternal: 0.40 pup: 0.12	Maternal: > 0.40 pup: 0.40	No effects. Post-natal death	[redacted] (1988b)

Summary of supplementary studies

Study/ species dose levels	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOEL	Reference
10-day dietary maternal toxicity; CF-1 mice;	- maternal: 0.08 (time-weighted)	- maternal: 0.24 (time-weighted)	Tremors, hunched posture, poor condition	[redacted] (1984g)
Exploratory acute oral toxicity; CF-1 mice of known genotype for P-glycoprotein	LD50 (+/+ genotype female mice): 28 mg/kg bw LD50 (+/- genotype female mice): 14 mg/kg bw	< 10 < 10	Tremors, bradypnea, decreased activity. Tremors, bradypnea, decreased activity, weight loss during first week	[redacted] 1997)
Exploratory oral toxicity; CF-1 / CD-1 mice (dose = 0.8 mg/kg bw for 4 days)	Results: All CF-1 mice showed tremors and ataxia, but 17% also showed dyspnea, lateral recumbence and coma (= sensitive to abamectin toxicity). All but one sensitive animal had no detectable P-glycoprotein in brain and small intestine. All insensitive CF-1 mice evaluated and all CD-1 mice had detectable P-glycoprotein levels. Control and treated CD-1 mice had similar levels of P-glycoprotein.			[redacted] (1994)

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

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