ABAMECTIN

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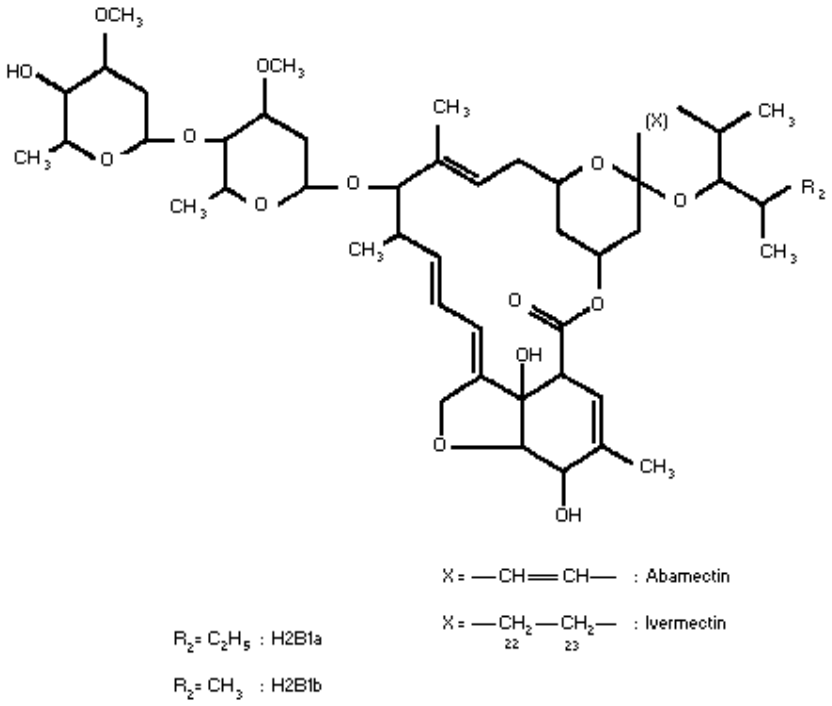
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Explanation

Abamectin (Fig. 1) is the International Organization for Standardization (ISO)–approved common name for a mixture of the components avermectin B_{1a} (≥ 80%) [(2a*E*,4*E*,8*E*)-(5′*S*,6*S*,6′*R*,7*S*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-6′-[(*S*)-*sec*-butyl]-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-arabino-hexopyranosyl)-3-*O*-methyl-α-L-arabino-hexopyranoside] and avermectin B_{1b} (≤ 20%) [(2a*E*,4*E*,8*E*)-(5′*S*,6*S*,6′*R*,7*S*,11*R*,13*S*,15*S*,17a*R*,20*R*,20a*R*,20b*S*)-5′,6,6′,7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-6′-isopropyl-5′,6,8,19-tetramethyl-17-oxospiro[11,15-methano-2*H*,13*H*,17*H*-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2′-[2*H*]pyran]-7-yl 2,6-dideoxy-4-*O*-(2,6-dideoxy-3-*O*-methyl-α-L-arabino-hexopyranosyl)-3-*O*-methyl-α-L-arabino-hexopyranoside] (International Union of Pure and Applied Chemistry), which has the Chemical Abstracts Service number 71751-41-2. Abamectin is a macrocyclic lactone product derived from the soil microorganism *Streptomyces avermitilis.* Because of the very similar biological and toxicological properties of the B_{1a} and B_{1b}

Fig. 1. Structural formulas of abamectin and ivermectin



Source: Annex 1, reference *91*

components, they can be considered to be equivalent. Abamectin is used as an insecticide and acaricide.

Abamectin was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, 1994, 1995 and 1997. In 1997, an acceptable daily intake (ADI) of 0–0.002 mg/kg body weight (bw) was established based on the no-observed-adverse-effect level (NOAEL) of 0.12 mg/kg bw per day for offspring toxicity in a two-generation reproductive toxicity study in rats, with the application of a reduced safety factor of 50 to account for the higher sensitivity of neonatal rats to abamectin, and the NOAEL of 0.24 mg/kg bw per day from a 1-year toxicity study in dogs, with the application of a safety factor of 100. The ADI was deemed to be appropriate for the sum of abamectin and its 8,9-*Z* isomer (a photodegraded product of abamectin), as the isomer was not expected to be of higher toxicity than abamectin. An acute reference dose (ARfD) was not established when abamectin was last evaluated by JMPR, as that evaluation pre-dated current JMPR guidance for the establishment of ARfDs for pesticides.

In 1996, the forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI of 0–0.001 mg/kg bw for abamectin used as a veterinary drug, based on the NOAEL of 0.12 mg/kg bw per day in the study on reproductive toxicity in rats and application of a safety factor of 100.

Abamectin was reviewed by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues (CCPR).

The majority of the toxicology studies were conducted with abamectin technical (also referred to as MK936 or avermectin B_1), which is a mixture of avermectin B_{1a} and avermectin B_{1b} (approximately 90:10). Some studies (mainly those requiring radiolabel) were conducted with either avermectin B_{1a} (also referred to as NOA422601) or avermectin B_{1b} (also referred to as NOA421704).

All critical studies were conducted in compliance with good laboratory practice (GLP), unless otherwise specified.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Differences in pharmacokinetics between two subpopulations of the CF-1 mouse were investigated by administering a single oral (gavage) dose of 0.1 or 0.2 mg/kg bw of ^{3}H-labelled abamectin (purity 96.2%) or emamectin (purity 95.5%) or 0.2 mg/kg bw of ^{3}H-labelled ivermectin (93.5% ivermectin B_{1a}, 2.3% ivermectin B_{1b}), formulated in sesame oil, to groups of up to 44 female wild-type (natural expression of *mdr1a* P-glycoprotein, +/+) and 44 female mutant (deficient expression of *mdr1a* P-glycoprotein, –/–) female mice (weighing 18.9–32.2 g). Groups of mice were terminated at timed intervals during the study, and the concentrations of radioactivity in blood, plasma, brain and bile were measured. Urine and faeces were collected from selected animals to provide preliminary information on the routes and rates of excretion of radioactivity.

The presence of radioactivity in the tissues of wild-type and mutant female CF-1 mice indicated systemic exposure to ^{3}H-labelled abamectin, emamectin or ivermectin following a single oral (gavage) dose of each of these test substances. In all dose groups, it was apparent that exposure in mutant mice was greater than that in wild-type mice.

Following a single oral dose of abamectin at 0.2 mg/kg bw, mutant mice developed adverse clinical signs, and the study was terminated prematurely. There were no significant clinical signs observed in the wild-type mice. In animals administered abamectin at a reduced dose level of 0.1 mg/kg bw, the wild-type and mutant animals displayed no clinical abnormalities. In a similar experiment with emamectin (0.1 mg/kg bw) and ivermectin (0.2 mg/kg bw), no clinical abnormalities were observed in any animals, with one exception.

Within each dose group and genotype, the profiles of radioactivity in blood and plasma were similar. Regardless of dose, the maximum concentrations (C_{max}) were higher, distribution was prolonged and elimination was slower in mutant animals compared with wild-type animals. When normalized for dose, maximum concentrations of radioactivity in blood and plasma and areas under the concentration–time curve from 0 to 72 hours ($AUC_{0–72}$) were relatively similar across the dose groups within the wild-type mice, as were those for mutant mice, where reported. However, the blood and plasma profiles of all of the test substances investigated showed a shift in the pattern of absorption into the systemic circulation between 4 and 12 hours, which in some cases amounted to a secondary peak (Table 1).

Concentrations of test substances were 40–160 times higher in the brains of mutant mice than in those of wild-type mice. Concentrations of radioactivity in the brains of wild-type animals were typically lower than concentrations seen in the blood and plasma, whereas in mutant mice, concentrations were typically higher (Table 2).

Excretion profiles for all animals showed that the primary route of excretion was via faeces. Urinary elimination was minimal in wild-type animals but was up to 5 times higher in mutant animals, although still a minor route of elimination compared with faecal elimination. The presence of radioactivity in bile indicates that there is some biliary excretion of these test substances, although the extent of elimination via bile could not be determined.

In view of the above, it is concluded that following administration of a single oral dose of abamectin, emamectin or ivermectin to wild-type or mutant CF-1 mice, exposure was greater in mutant mice than in wild-type mice. Distribution and elimination also appeared to be slower, with concentrations in the brain tissue up to 160 times higher in mutant mice than in wild-type mice. The exposure profiles of the three test substances were broadly similar. Excretion profiles showed that the primary route of excretion was via faeces.

Table 1. Derived pharmacokinetic parameters for blood and plasma

Dose group	Animal type	C_{max} (µg equiv/g)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0–72}$ (h·µg/g)	$AUC_{0–\infty}$ (h·µg/g)	Study duration (h)
Blood							
Abamectin 0.2 mg/kg bw	Wild-type	0.028	4	NR	0.51	0.52	0–96
	Mutant	NR	NR	NC	NC	NC	0–96[a]
Abamectin 0.1 mg/kg bw	Wild-type	0.010	4	NR	0.18	0.19	0–120
	Mutant	0.023	12	NR	0.66	0.73	0–120
Emamectin 0.1 mg/kg bw	Wild-type	0.013	8	18.6	0.22	0.23	0–96
	Mutant	0.019	12	37.6	0.77	0.89	0–96
Ivermectin 0.2 mg/kg bw	Wild-type	0.015	8	NR	0.29	0.29	0–72
	Mutant	0.030	8	NR	1.32	1.32	0–72
Plasma							
Abamectin 0.2 mg/kg bw	Wild-type	0.050	4	NR	0.93	0.95	0–96
	Mutant	NR	NR	NC	NC	NC	0–96[a]
Abamectin 0.1 mg/kg bw	Wild-type	NR	NR	NC	NC	NC	0–120
	Mutant	NR	NR	NC	NC	0.19	0–120
Emamectin 0.1 mg/kg bw	Wild-type[b]	0.026	8	19.5	0.43	0.43	0–96
	Mutant	0.034	12	NR	1.27	1.41	0–96
Ivermectin 0.2 mg/kg bw	Wild-type	0.032	8	NR	0.55	0.55	0–72
	Mutant	0.056	8	NR	2.40	2.41	0–72

$AUC_{0–72}$: area under the concentration–time curve from 0 to 72 hours; $AUC_{0–\infty}$: area under the concentration–time curve from time 0 to infinity; bw: body weight; C_{max}: maximum concentration; NC: not calculable; NR: not reported; $t_{½}$: half-life; T_{max}: time to reach the maximum concentration

[a] Study terminated prematurely.

[b] Female 243 excluded.

Source: Gledhill (2008)

Table 2. Pharmacokinetic parameters in brains of mice

Dose group	Animal type	C_{max} (µg equiv/g)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0–72}$ (h·µg/g)	$AUC_{0–\infty}$ (h·µg/g)	Study duration (h)
Abamectin 0.2 mg/kg bw	Wild-type	0.006	4	15.0	0.144	0.144[a]	0–96
	Mutant	NR	NR	NC	NC	NC	0–96[b]
Abamectin 0.1 mg/kg bw	Wild-type	0.002	4	NR	0.068	0.081	0–120
	Mutant	0.077	12	NR	2.532	2.760	0–120
Emamectin 0.1 mg/kg bw	Wild-type[c]	0.002	12	30.3	0.049	0.052	0–96
	Mutant	0.167	24	31.4	7.310	8.405	0–96
Ivermectin 0.2 mg/kg bw	Wild-type	0.006	0.5	NR	0.070	0.070	0–72
	Mutant	0.16	24	NR	7.095	7.095	0–72

$AUC_{0–72}$: area under the concentration–time curve from 0 to 72 hours; $AUC_{0–\infty}$: area under the concentration–time curve from time 0 to infinity; bw: body weight; C_{max}: maximum concentration; NC: not calculable; NR: not reported; $t_{½}$: half-life; T_{max}: time to reach the maximum concentration

[a] AUC up to 72 hours only.

[b] Study terminated prematurely.

[c] Female 243 excluded.

Source: Gledhill (2008)

A 0.2 mg/kg bw dose of abamectin caused adverse clinical signs in mutant mice. In similar experiments with abamectin (0.1 mg/kg bw), emamectin (0.1 mg/kg bw) or ivermectin (0.2 mg/kg bw), the majority of animals displayed no adverse clinical signs.

The study was GLP compliant, and a quality assurance (QA) statement was attached (Gledhill, 2008).

Rats

Fifty-three rats of each sex (CRCD strain, body weights approximately 295 g [male] and 217 g [female]) were divided into four groups and treated orally, by gavage, with [5-^{3}H]avermectin B_{1a} (batch no. L-676,895-01S15, radiochemical purity 99.4%), [3,7,11,13,23-^{14}C]avermectin B_{1a} (batch no. L-676,895-29T01, radiochemical purity 99%) or unlabelled avermectin B_{1a} (batch no. L-676,895-00P76) dissolved in sesame oil, according to the schedule shown in Table 3. A further nine rats of each sex, treated with sesame oil alone, acted as controls.

Subgroups of three males and three females within each group were caged together, by sex, for the collection of urine and faeces. Urine and faeces were collected at 24-hour intervals until sacrifice. At necropsy, bone, brain, fat, gonads, heart, kidney, liver, gastrointestinal tract, lung, blood, muscle, spleen and residual carcass (less skin and tail) were collected. The same tissues from the animals within each subgroup were combined, and the total weights were determined. Blood and urine samples and 1:4 water homogenates of each tissue (0.5 mL) were weighed and combusted in a Packard 306 sample oxidizer, and the $^{3}H_2O$ and $^{14}CO_2$ were trapped and then counted for 10 minutes in a Packard scintillation spectrometer to determine total radioactivity.

All samples were combusted in duplicate, except for brain, lung, bone, spleen and heart, which were weighed and combusted directly.

The residue levels and depletion rates in the groups treated with tritiated avermectin B_{1a} were comparable with the values obtained from rats treated with ^{14}C-labelled avermectin B_{1a}. Therefore, it was concluded that tritium labelling at the 5-position of avermectin B_{1a} was stable.

Table 3. Treatment and sacrifice schedule

Group	No. of animals (M + F)	Test article	Specific activity (µCi/mg)[a]	Dose level × no. of doses	Sacrifice schedule
A	12 + 12	[^{3}H]Avermectin B_{1a}	350	1.42 mg/kg bw (M) × 1 1.38 mg/kg bw (F) × 1	3M + 3F at 1, 2, 4 and 7 days after dose
B	12 + 12	[^{3}H]Avermectin B_{1a}	350	0.142 mg/kg bw (M) × 1 0.138 mg/kg bw (F) × 1	3M + 3F at 1, 2, 4 and 7 days after dose
C	17 + 17	Avermectin B_{1a} [^{3}H]Avermectin B_{1a}	Unlabelled 350	0.142 mg/kg bw (M) × 15[b] 0.138 mg/kg bw (F) × 15[b]	3M + 3F at 1, 2, 4 and 7 days after last dose
D	12 + 12	[^{3}H]Avermectin B_{1a} [^{14}C]Avermectin B_{1a}	33 16.4	1.42 mg/kg bw (M) × 1 1.38 mg/kg bw (F) × 1	3M + 3F at 1, 2, 4 and 7 days after dose
E	3 + 3	Sesame oil	–	0.55 mL/rat (M) × 1 0.40 mL/rat (F) × 1	3 M + 3F 7 days after dose
F	6 + 6	Sesame oil	–	0.55 mL/rat per day (M) × 15 0.40 mL/rat per day (F) × 15	3M + 3F 7 days after last dose

bw: body weight; F: female; M: male

[a] 1 µCi = 37 kBq.

[b] Fourteen daily doses of unlabelled material followed by a single dose of [^{3}H]avermectin B_{1a}.

Source: Alvaro et al. (1984)

The data reported for dose accountability were calculated on the basis of the total dose given to each group and are therefore average values from animals sacrificed 1, 2, 4 and 7 days after dosing. The total percentage of the dose accounted for in the analysed samples for each group ranged from 85.1% to 95.1% for tritium and from 94.5% to 94.9% for ^{14}C. Similarly, the excretion data reported were calculated on the basis of the total dose given to each group, whereas the daily elimination of the dose should have been calculated for each subgroup. Therefore, the excretion data in percentage of the dose for each subgroup sacrificed at intervals up to 7 days after dosing were recalculated based on the reported values of the daily elimination of the dose in micrograms. The total rate of excretion was very similar for both sexes and was independent of the dose level and treatment regimen. Excretion was moderately rapid, with 80–101% of the dose excreted within 96 hours. The route of excretion was independent of the dose level and sex. The dose was almost completely excreted, almost entirely via the faeces (86.8–104% of the dose). Urinary excretion was extremely low, accounting for 0.5–1.4% of the dose.

Mean residue levels in sampled tissues from male and female rats were highest in all tissues 24 hours after treatment and were dose dependent, as the residues in the high-dose groups (1.38/1.42 mg/kg bw) were approximately 10-fold higher than those in the low-dose groups (0.138/0.142 mg/kg bw). In contrast, the depletion rates were independent of dose level, as the tissue half-lives were comparable between the dose levels. There were no substantive differences in the depletion half-lives among the tissues. Most of the calculated half-lives were within the range 1.2 ± 0.3 days, although brain and testes showed greater variability. The highest residue levels at 24 hours in tissues, excluding the gastrointestinal tract, occurred in fat (2.89–5.30 and 0.16–0.32 parts per million [ppm] equivalents for the high and low dose levels, respectively). The brain contained the lowest residues (0.07–0.13 and 0.01 ppm equivalents for the high and low dose levels, respectively). In most instances, residue levels in the kidney were higher than the levels in the liver. Pretreatment for 14 days with unlabelled avermectin B_{1a} at a dose of 0.138/0.142 mg/kg bw had no effect on either the residue levels or depletion rates, compared with a single administration at the same dose level. The tissue residue levels in female rats were generally higher than the corresponding residue levels in male rats.

In view of the above, it can be concluded that avermectin B_{1a} is absorbed from the gastrointestinal tract and is distributed throughout all major tissues and organs sampled. It is rapidly eliminated from the body, almost exclusively in the faeces, and there is no evidence for tissue accumulation on repeated administration. With the exception of dose dependence for tissue residue levels, the toxicokinetic/ pharmacokinetic profile is not influenced by sex, dose level or treatment regimen.

The study was not GLP compliant, and no QA statement was attached (Alvaro et al., 1984).

In another study, [23-^{14}C]avermectin B_{1a} was administered as a single oral dose at two dose levels – i.e. 0.5 mg/kg bw (low dose) and 5 mg/kg bw (high dose) – to several groups of male and female rats (Hanlbm: WIST (SPF)). Biliary excretion was investigated after single oral administration at the low dose level in bile duct–cannulated male and female rats. Additionally, [23-^{14}C]avermectin B_{1a} was administered intravenously at the low dose level to male rats in order to obtain additional insight into the routes of excretion.

At the time of dosing with radiolabelled avermectin B_{1a}, the animals of groups B1, D1, F1–F4 and L1 had a body weight of approximately 200 g, corresponding to 7 and 11 weeks of age for males and females, respectively. For the biliary excretion investigation (groups G1 and G2), the animals had a body weight of approximately 250 g and 210 g, corresponding to 9 and 12 weeks of age for males and females, respectively.

For administration, [23-^{14}C]avermectin B_{1a} was dissolved in a 3:2 volume per volume (v/v) mixture of polyethylene glycol 200/ethanol at different concentrations: intravenous, about 1 mg/mL; and oral, 0.2 mg/mL and 2 mg/mL for the low and high dose levels, respectively. For the oral administration, each animal received 0.5 mL of the administration solution by stomach tube. The

intravenous administration was performed by injection into the tail vein with a volume of 0.1 mL each.

Groups B1/D1. After single oral administration of [23-^{14}C]avermectin B_{1a}, the urine (0–168 hours), faeces (0–168 hours), expired air (0–48 hours) and serial blood (0–72 hours) were individually collected, and the radioactivity was determined by liquid scintillation counting. Expired air was collected only from animals of the high-dose group (D1). Seven days after dosing, the animals were killed by exsanguination after anaesthesia with carbon dioxide. The terminal blood was collected; whole blood was separated into plasma and red blood cells by centrifugation. The tissues and organs were removed by dissection and taken for radiometry.

Groups G1/G2. [23-^{14}C]Avermectin B_{1a} was administered to bile duct–cannulated male and female rats. During the experimental period, artificial bile fluid was infused into the duodenum of the animals. Urine (0–48 hours), faeces (0–48 hours) and bile fluid (0–48 hours) were individually collected, and the radioactivity was determined by liquid scintillation counting. Two days after dosing, the animals were killed, and the gastrointestinal tract, including contents, and the remaining carcass were collected for radiometry.

Groups F1–F4. At four different time points after administration, three animals each were killed by exsanguination, and terminal blood was collected. Plasma was separated from whole blood by centrifugation. The time points selected were based on the blood kinetics as determined in groups B1 and D1. After sacrifice, the tissues and organs were removed by dissection and analysed for residual radioactivity.

Group L1. [23-^{14}C]Avermectin B_{1a} was administered intravenously to male rats. Urine and faeces were collected and analysed for radioactivity. At two time points – i.e. 6 hours (t_1) and 24 hours (t_2) – after administration, two animals were sacrificed. One animal was immediately frozen as a whole; the frozen carcass was embedded in gelatine solution and frozen as a block. Whole-body sections were taken at different body levels, and the radioactivity in the tissues, organs and areas of the gastrointestinal tract was determined by radioluminography.

From the second animal of each sampling time point, the gastrointestinal tract was removed, and the loops of the intestinal tract were carefully separated without damaging the intestinal tract. Thereafter, the gastrointestinal tract was placed in a straight line on a framed specimen stage, which was submerged in a mixture of solid carbon dioxide and hexane at approximately −80 °C. The gastrointestinal tract was embedded in gelatine solution and frozen as a block. Sections were taken at different levels, and the radioactivity in the contents and wall of the gastrointestinal tract was determined by radioluminography.

The details of this experiment are summarized in Table 4.

The test substance was stable in the administration solution, as indicated by a radiochemical purity greater than 96% (determined by thin-layer chromatography) at the time points of dosing for each group.

After a single oral administration, the blood concentration–time curves demonstrated a moderately rapid absorption from the gastrointestinal tract into the systemic circulation at both dose levels, independent of sex. The maximum concentration (C_{max}) in blood at both dose levels and in both sexes was reached within 4–8 hours after administration, accounting for about 0.06 ppm and 0.6 ppm [23-^{14}C]avermectin B_{1a} equivalents for the low and high doses, respectively. After reaching the maximum concentration, residues in blood depleted within 15–27 hours to half of the maximum concentration. The area under the concentration–time curve (AUC) values were similar between the sexes – i.e. 1.2 h·µg/g and 1.3 h·µg/g at the low dose and 17 h·µg/g and 18 h·µg/g at the high dose for males and females, respectively. Maximum blood concentrations and AUC values increased in a proportional fashion, with the dose level indicating a dose-independent systemic bioavailability (Table 5).

Table 4. Summary of dosing and sampling regimen for each group treated with [23-^{14}C]avermectin B_{1a}

Group	Number of each sex	Dose and route	Sampling regimen
B1	4 males 4 females	Low dose oral	Collection of urine and faeces at different time points, blood kinetics 0–48 h, and various tissues 7 days after administration
D1	4 males 4 females	High dose oral	Collection of urine, faeces and expired air at different time points, blood kinetics 0–72 h, and various tissues 7 days after administration
F1	12 males	Low dose oral	Collection of various tissues at different time points, i.e. 6, 24, 48 and 72 h after administration
F2	12 females	Low dose oral	Collection of various tissues at different time points, i.e. 6, 24, 48 and 72 h after administration
F3	12 males	High dose oral	Collection of various tissues at different time points, i.e. 8, 24, 48 and 72 h after administration
F4	12 females	High dose oral	Collection of various tissues at different time points, i.e. 8, 24, 48 and 72 h after administration
G1	6 males	Low dose oral	Collection of urine, bile and faeces at different time points up to 48 h after administration and gastrointestinal tract and remaining carcass after sacrifice
G2	6 females	Low dose oral	Collection of urine, bile and faeces at different time points up to 48 h after administration and gastrointestinal tract and remaining carcass after sacrifice
L1	4 males	Low dose intravenous	Collection of urine and faeces, whole-body radioluminography and radioluminography of the gastrointestinal tract at two different time points, i.e. 6 and 24 h after administration

Source: Hassler (2001)

Table 5. Blood kinetic parameters of [23-^{14}C]avermectin B_{1a} after single oral administration

	Group B1: 0.5 mg/kg bw		Group D1: 5 mg/kg bw	
Parameter	**Males**	**Females**	**Males**	**Females**
C_{max} (ppm)	0.057	0.049	0.616	0.522
T_{max} (h)	4	8	8	8
$T_{max/2}$ (h)	19	24	26	35
AUC (h·µg/g)	1.2	1.3	17.0	18.0

bw: body weight; C_{max}: maximum concentration in blood; ppm: parts per million; T_{max}: time to reach C_{max}; $T_{max/2}$: time to decline to half C_{max}
Source: Hassler (2001)

The apparent extent of absorption, based on the amount excreted via the kidneys and the bile, was determined to be 3–5% of the dose. However, the real extent of absorption is significantly higher based on consideration of the tissue residue data and comparison of urinary excretion following oral or intravenous administration. The total tissue residues determined at the time point of the maximum concentration – i.e. 6 and 8 hours after administration – accounted for about 50% of the dose for both dose levels. The comparison of urinary excretion after oral and intravenous administration results in a ratio that is equivalent to the biovailability of a test substance. A bioavailability factor of 0.86 was calculated for abamectin, which justifies the assumption of almost complete oral absorption.

The orally administered doses were excreted moderately rapidly. The major part of the dose was excreted via the faeces – i.e. more than 92% of the dose at both dose levels and for both sexes within 7 days after administration. Only very small amounts were renally excreted, accounting for about 1%, essentially independent of sex and dose level. At the low dose level, low amounts of the absorbed dose – i.e. 5% in males and 3% in females – were also eliminated via bile and ultimately excreted with the faeces. The rate of excretion was slightly slower in females than in males. Whereas males excreted 81% and 73% of the dose within 48 hours after administration of the low and high doses, respectively, females showed an excretion of 69% and 48% of the low and high doses, respectively, within the same period. The excretion of the absorbed test substance via expired air was insignificant. Only 0.01% of the dose was determined in the expired air within 48 hours after administration of the high dose.

The rates and routes of excretion were essentially independent of the dose level, the route of administration and the sex of the animals.

The non-biliary excretion into the gastrointestinal tract was investigated after intravenous administration of [23-^{14}C]avermectin B_{1a} to male rats at the low dose of 0.5 mg/kg bw. At two different time points – i.e. 6 and 24 hours after administration – the gastrointestinal tract of one animal was removed after sacrifice, and the concentration of radioactivity was determined in different parts of the gastrointestinal tract by radioluminography. Six hours after intravenous administration, the contents of the stomach revealed, as expected, only a low concentration of radioactivity (0.016 ppm [23-^{14}C]avermectin B_{1a} equivalents), whereas the secretory epithelium of the stomach wall revealed a concentration of 0.380 ppm equivalents. The intestinal wall from the duodenum to the rectum showed concentrations of radioactivity in the range of 0.250–0.800 ppm [23-^{14}C]avermectin B_{1a} equivalents, indicating an excretion of [23-^{14}C]avermectin B_{1a} and/or its metabolites into the intestinal tract. The contents of the gastrointestinal tract revealed increasing concentrations from the duodenum to the ileum, accounting for 1–4 ppm equivalents. Thereafter, the concentration of radioactivity in the contents decreased from the colon (1.9 ppm equivalents) through to the rectum (0.04 ppm equivalents). The concentration in the rectum at the last position was significantly lower than the corresponding concentration in the wall of the rectum. Twenty-four hours after intravenous administration, the concentration in the intestinal wall decreased to levels in the range of 0.15–0.40 ppm equivalents. The contents of the gastrointestinal tract showed increasing concentrations from 0.5 ppm equivalents in the duodenum up to 3.2 ppm equivalents at the last determined position in the rectum. The high concentration of radioactivity in the stomach contents 24 hours after administration may be due to oral uptake of excreted faeces (i.e. sacrophagy of rodents).

After single oral administration of the low dose, all selected tissues and organs achieved the highest residue levels 6 hours after administration, with the exception of fat, where the residues reached the maximum level 24 hours after administration. The highest residues were found in adrenals, fat, liver and pancreas, in the range 0.67–1.05 ppm [23-^{14}C]avermectin B_{1a} equivalents. All other selected tissues and organs showed maximum residue levels between 0.1 and 0.6 ppm equivalents, except for testes (0.04 ppm equivalents) and brain (0.01 ppm equivalents). The maximum residue levels for males and females were similar.

After reaching their maximum concentrations, the tissue residues depleted following monophasic first-order kinetics, with half-lives in the range of 12–17 hours for males and 13–33 hours for females. Within 7 days after administration, the tissue residue levels were low. Based on the slowest depletion rate, the highest residue levels were determined in fat, accounting for 0.065 and 0.073 ppm [23-^{14}C]avermectin B_{1a} equivalents in males and females, respectively.

After administration of the high dose, the residues were about 10 times higher, corresponding to the 10-fold higher dose level. The profile of the maximum levels was essentially the same as that determined at the low dose. The highest residue levels were determined in fat, accounting for 11.3 and 13.5 ppm equivalents for males and females, respectively, followed by adrenals (10.3 and 10.9 ppm equivalents, respectively), liver (9.4 and 10.6 ppm equivalents, respectively) and pancreas (6.3 and 7.6 ppm equivalents, respectively). The calculated depletion half-lives, based on monophasic first-order kinetics, ranged from 12 to 20 hours in males and from 20 to 35 hours in females. The residues

declined within 7 days after administration to levels not exceeding 0.1 ppm [23-^{14}C]avermectin B_{1a} equivalents, except in fat, where residue levels of 0.95 ppm equivalents in males and 1.15 ppm equivalents in females were determined.

Approximately 50% of the administered dose was totally recovered in selected tissues and organs 6 and 8 hours after administration of the low and high doses, respectively. The absorbed test substance and/or its metabolites were widely distributed within the body. After reaching the maximum residue concentration, the residues depleted to approximately 1% and 2% of the dose within 168 hours in males and females, respectively.

The residue levels in tissues and organs determined by whole-body radioluminography 6 and 24 hours after the single intravenous administration at the low dose confirm the wide and nonspecific distribution of the radioactivity.

The concentrations of radioactivity in the contents of the small intestine, appendix and large intestine were 1.8, 2.8 and 3.0 ppm [23-^{14}C]avermectin B_{1a} equivalents, respectively. The presence of radioactivity in the contents of the intestinal tract after intravenous administration, especially at a high concentration, confirms the assumption of a non-biliary excretion of absorbed avermectin B_{1a} into the intestinal tract and ultimately excretion in the faeces.

Comparison of the tissue residue levels after intravenous administration with those in rats that were given a similar oral dose shows that the residue levels were similar at the corresponding time points, which indicates almost complete absorption after oral administration.

It can be concluded that after oral administration to rats, the majority of avermectin B_{1a} was almost completely absorbed and then eliminated predominantly by non-biliary excretion into the gastrointestinal tract before excretion with the faeces. The rates and mechanisms of oral absorption and subsequent excretion were independent of the dose level and the sex; however, the depletion of tissue residues was more rapid in males than in females, by approximately 2-fold.

The study was GLP compliant, and a QA statement was attached (Hassler, 2001).

In another study, single oral doses of [23-^{14}C]avermectin B_{1b} were administered at two dose levels – i.e. 0.5 mg/kg bw (low dose) and 5 mg/kg bw (high dose) – to two groups of male and female rats (Hanlbm: WIST (SPF)) weighing approximately 200 g, corresponding to 7 and 9 weeks of age for the males and females, respectively.

Avermectin B_{1b} was dissolved in a 4:2 v/v mixture of polyethylene glycol 200/ethanol at a concentration of 0.2 mg/mL and 2 mg/mL for the low and high dose levels, respectively. Each animal received 0.5 mL of the administration solution by stomach tube. Specimen collections were made from the individual animals at the following time intervals/time points after administration:

- *Urine*: 0–6, 6–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours;
- *Faeces*: 0–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 hours;
- *Serial blood*: 0.5, 1, 2, 4, 8, 12, 24 and 48 hours.

Seven days after dosing, the animals were killed. The terminal blood was collected; whole blood was separated into plasma and red blood cells by centrifugation. The tissues and organs, including the remaining carcass, were taken for examination of residual radioactivity.

Urine and faeces pools according to sex and animal group were prepared by mixing equal aliquots of the collected samples from each animal.

Radioactivity in all samples was measured by liquid scintillation counting. The metabolite pattern in urine and faeces was determined using thin-layer chromatography. Metabolic profiles in these excreta samples were analysed by high-performance liquid chromatography (HPLC).

After a single oral administration, the blood concentration–time curves for both sexes demonstrate a moderately rapid absorption from the gastrointestinal tract into the systemic circulation at both dose levels.

Table 6. Blood kinetic parameters of [23-^{14}C]avermectin B_{1b} after a single oral administration to rats

	Group B1: 0.5 mg/kg bw		Group D1: 5 mg/kg bw	
Parameter	**Males**	**Females**	**Males**	**Females**
C_{max} (ppm)	0.044	0.048	0.49	0.42
T_{max} (h)	8	4	4	8
$T_{max/2}$ (h)	9	13	14	21
AUC (h·µg/g)	0.72	0.86	9.0	11.5

AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration in blood; ppm: parts per million; T_{max}: time to reach C_{max}; $T_{max/2}$: time to decline to half C_{max}
Source: Briswalter (2003a)

The parameters of the kinetics of [23-^{14}C]avermectin B_{1b} in blood are summarized in Table 6.

After a single oral administration at the low and high dose levels – i.e. 0.5 and 5 mg/kg bw (groups B1 and D1) – the apparent absorption of avermectin B_{1b}, based on the radioactivity determined in urine and tissue residues, accounted for only 6–7% of the dose. Very low levels (4–5% of the administered dose) were determined in the urine over the experimental period of 168 hours. However, comparison with avermectin B_{1a} (Hassler, 2001) indicates that the actual extent of absorption is significantly higher.

The rates and routes of excretion were essentially independent of the dose level and the sex of the animals.

At the low dose level, only 5% and 4% of the dose were renally excreted within 168 hours after administration to male and female rats, respectively. The majority of the dose was excreted via the faeces, accounting for 94% and 91% of the dose in male and female rats, respectively. Female rats showed a slightly slower excretion compared with male rats. Whereas male rats excreted 94% of the dose within 48 hours of administration, female rats excreted 82% of the dose within the same period. This sex difference in the rate of excretion is comparable with that seen with avermectin B_{1a} in rats (Hassler, 2001). However, the residues in excised tissues and carcass 168 hours after administration accounted in total for only 2% of the dose for both male and female rats.

The rates and routes of excretion after single oral administration of the 10-fold higher high dose (5 mg/kg bw) were very similar to those following administration of the low dose. Approximately 4% of the dose was excreted with the urine in both sexes, and 89% and 92% of the dose were excreted with the faeces in male and female rats, respectively. Again, a slightly slower excretion was observed in female rats. Whereas male rats excreted 89% of the dose within 48 hours after administration, female rats excreted 70% of the dose within the same period.

After absorption, the test substance was widely distributed in the whole body of the animals. At the low dose (0.5 mg/kg bw), the tissue residue levels 7 days after oral administration were low. The highest residue levels were found in fat, accounting for 0.09 and 0.16 ppm [23-^{14}C]avermectin B_{1b} equivalents in male and female rats, respectively. All other tissues and organs showed residue levels below 0.04 ppm equivalents. After administration at the high dose (5 mg/kg bw), the residue levels were about 10 times higher, corresponding to the 10-fold higher dose. The tissue distribution was very similar to that observed at the low dose. The highest residue levels were found in fat, accounting for 1.4 and 1.6 ppm [23-^{14}C]avermectin B_{1b} equivalents in male and female rats, respectively. All other tissues and organs showed residue levels below 0.3 ppm equivalents.

It can be concluded that after oral administration to rats, [23-^{14}C]avermectin B_{1b} was almost completely absorbed and then eliminated predominantly by non-biliary excretion into the gastrointestinal tract and ultimately excreted with the faeces. The rates and mechanisms of absorption and excretion were independent of the dose level and the sex, with the exception of a slower rate of

excretion in females. The absorption, distribution, degradation and excretion of [23-^{14}C]avermectin B_{1b} and [23-^{14}C]avermectin B_{1a} were essentially similar.

The study was GLP compliant, and a QA statement was attached (Briswalter, 2003a).

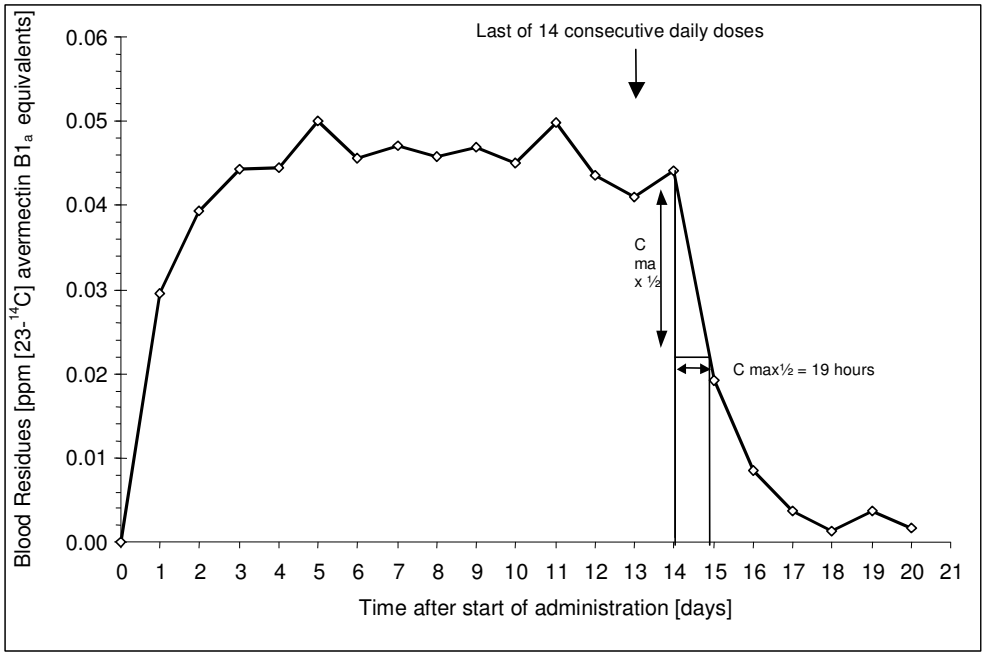
To examine the distribution and clearance of [23-^{14}C]avermectin B_{1a} after multiple dosing, [23-^{14}C]avermectin B_{1a} was orally administered to 16 female rats. The rats received up to 14 consecutive daily doses at a nominal dose of 0.5 mg/kg bw. Four animals each were assigned to subgroups (T1–T4), which were sacrificed at four different time points after the start of administration, and the avermectin B_{1a}–related residues were determined in tissues and organs. The excreted radioactivity was determined in urine and faeces of subgroup T4 at daily intervals. The dosing, sampling and sacrifice time points are summarized as follows:

- *Dosing*: days 0–13;
- *Sacrifice of subgroups*: T1 = day 1, T2 = day 7, T3 = day 14, T4 = day 20;
- *Serial blood sampling T4*: daily on days 1–20;
- *Excreta T4*: daily on days 0–20.

At the time of dosing with radiolabelled avermectin B_{1a}, the rats had a body weight of approximately 180 g and were about 9 weeks of age. The test substance avermectin B_{1a} was dissolved in a 3:2 v/v mixture of polyethylene glycol 200/ethanol at a concentration of about 0.2 mg/mL. Each animal received 0.5 mL of the administration solution by stomach tube. At the defined time points specified above, blood and excreta were collected from subgroup T4. At terminal sacrifice, the tissues and organs were taken for examination of residual radioactivity.

A plateau of blood concentration was reached 3 days after the start of dosing, at about 0.045 ppm avermectin B_{1a} equivalents. One day after the last of 14 consecutive daily doses, the blood residue levels declined rapidly, reaching half the maximum concentration within 19 hours (Fig. 2).

Fig. 2. Profile of [23-^{14}C]avermectin B_{1a} ppm equivalents in blood



Source: Hassler (2003)

During the dosing period – i.e. 14 consecutive daily doses – a steady state in terms of excretion was reached 2 days after the first administration. The majority of the administered test substance (96%) was excreted 7 days after the last of 14 consecutive daily doses. Less than 1% of the total dose remained in the tissues and organs at the end of the experimental period. The administered test substance was excreted almost exclusively with the faeces, accounting for 96% of the total dose. An insignificant amount (0.8% of the total dose) was excreted with the urine.

A plateau of the tissue residue levels was reached after 7 days of multiple dosing for all selected tissues and organs, except for thyroid, which showed an almost linear increase of the residue levels during the dosing period. The highest residue levels were found in fat, accounting for 2.6 ppm [23-^{14}C]avermectin B_{1a} equivalents, followed by adrenals (0.77 ppm equivalents), pancreas (0.66 ppm equivalents), liver (0.63 ppm equivalents) and thyroid (0.60 ppm equivalents). All other tissues and organs reached maximum levels below 0.5 ppm [23-^{14}C]avermectin B_{1a} equivalents. The calculated half-lives for the depletion of the residual radioactivity from tissues and organs, assuming monophasic first-order kinetics, were in the range of 1–3 days for all selected tissues and organs.

The residue levels in tissues and organs 7 days after the last of 14 consecutive daily doses revealed a similar distribution pattern and were only 2–4 times higher than the tissue residue levels 7 days after a single oral administration of [23-^{14}C]avermectin B_{1a} at the low dose (0.5 mg/kg bw), except for fat, which showed a 7 times higher residue level.

In summary, the rates and routes of excretion and the tissue distribution did not change upon multiple dosing compared with single dosing. With the exception of the thyroid, the tissue residues reached a plateau after 7 days of dosing, and for all tissues, the residues declined very rapidly after the dosing period.

The study was GLP compliant, and a QA statement was attached (Hassler, 2003).

1.2 Biotransformation

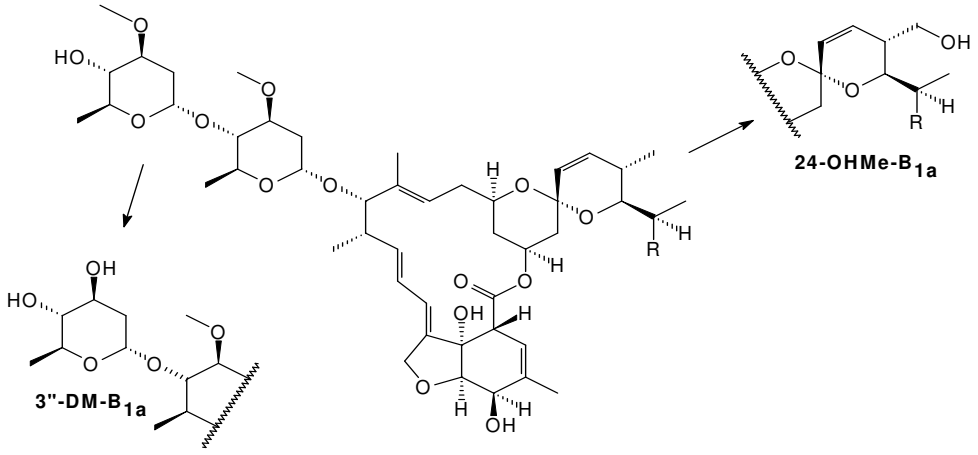
Selected tissue samples derived from the study by Alvaro et al. (1984) (described in section 1.1 above) were analysed for parent avermectin B_{1a}. Liver, kidney, muscle and fat samples were analysed by an indirect method, reverse isotope dilution assay. The residues were separated and purified by reversed-phase HPLC radioactivity profiling and quantified by calculation. Radioactivity in liquid samples was measured by liquid scintillation counting.

Metabolite structures were identified by nuclear magnetic resonance spectrometry and fast atom bombardment mass spectrometry. The isolated metabolites were identified as 24-hydroxymethyl-avermectin B_{1a} (24-OHMe-B_{1a}) and 3″-*O*-desmethyl-avermectin B_{1a} (3″-DM-B_{1a}) and were used as reference substances. Comparison of the HPLC profiles of tissue extracts from rats dosed with both ^{3}H- and ^{14}C-labelled avermectin B_{1a} demonstrated that the profiles were identical. All radioactivity peaks had a constant ^{3}H/^{14}C ratio and the same percentage of recovered radioactivity.

Parent avermectin B_{1a} as a percentage of the total residue was not dependent on dose level. The half-lives of avermectin B_{1a} in tissues of male rats (0.6–1.0 day) were slightly less than in tissues of female rats (0.9–1.0 day). The proportion of avermectin B_{1a} as a percentage of the total residue was similar for both ^{3}H- and ^{14}C-labelled avermectin B_{1a}. Avermectin B_{1a} as a percentage of total residue was lower in tissues from males than in tissues from females. Pretreatment with unlabelled avermectin B_{1a} for 14 days exerted a minimal or negligible effect on tissue residue levels. The residues in liver, kidney, muscle and fat were characterized by HPLC. In addition to unchanged avermectin B_{1a}, two metabolites were observed and subsequently identified. Identification was accomplished by comparison of the retention times of the metabolites and reference substances and also by co-chromatography with reference substances. The reference substances were isolated from rat liver microsome incubation of avermectin B_{1a}. The metabolites were identified as 24-OHMe-B_{1a} and 3″-DM-B_{1a}. The metabolic pathway is illustrated in Fig. 3.

Quantitative results for the metabolites were reported only for some treatment and sampling regimens. In females of the group treated with a single dose of 1.4 mg/kg bw and sacrificed 2 days

Fig. 3. Metabolic pathway of avermectin B_{1a} formed in rats in vivo



Source: Maynard, Wislocki & Lu (1986)

after treatment, 24-OHMe-B_{1a} and 3″-DM-B_{1a} accounted for 6% and 24%, respectively, of the total residue in liver and for 3% and 24%, respectively, in kidney. Unchanged avermectin B_{1a} accounted for 46% of the total residue in liver and 55% in kidney. The metabolic profile in muscle was similar to the profiles in liver and kidney, but no quantitative results were reported. The metabolic profile in fat was different from that in the other tissues. A non-polar fraction was detected that was assumed to consist of non-polar conjugates of 24-OHMe-B_{1a} and 3″-DM-B_{1a}. Other unknown minor metabolite fractions occurred on the chromatograms presented in the report, but no quantitative data were reported.

The use of a tritium label on avermectin B_{1a} is appropriate for animal metabolism studies. Unchanged parent avermectin B_{1a} as a proportion of total residues is not dose dependent but is slightly lower in male rats than in female rats. The tissue half-lives of avermectin B_{1a} are slightly lower in male rats than in female rats. Avermectin B_{1a} and/or its metabolites do not accumulate in liver, kidneys, muscle or fat on repeated administration of a low dose.

The study was not GLP compliant (Maynard, Wislocki & Lu, 1986).

In a multiple oral dosing study with [23-^{14}C]avermectin B_{1a} in female rats (Hassler, 2003), the metabolite pattern of urine and faeces investigated at three different time intervals during the dosing period – i.e. days 0–1, days 6–7 and days 13–14 – was essentially identical for all of the analysed time intervals. The major part of the administered test substance was excreted with the faeces as unchanged parent, accounting for about 40% of the daily dose.

From the study by Hassler (2001) (described in section 1.1 above), selected tissue samples were analysed to identify and quantify individual metabolites to derive the metabolic pathways of orally administered [23-^{14}C]avermectin B_{1a} at a dose of 0.5 or 5 mg/kg bw. Urine, faeces, bile, fat and muscle specimens from male and female rats generated in that study were separately pooled and used for analysis in the present study.

Urine, faeces and bile pools were prepared, according to sex and animal group, by mixing equal aliquots of the collected samples from each animal (0–168 hours or 0–48 hours for bile duct–cannulated animals). Fat and muscle pools were prepared by mixing the whole samples (remaining after determination of the total radioactive residues) from animals of subgroups at the designated time points (8, 24 and 48 hours). Radioactivity in all samples was measured by liquid scintillation counting, and the quantitative metabolite pattern was determined by thin-layer chromatography. Metabolites were isolated by chromatographic techniques, such as solid-phase extraction followed by

HPLC and thin-layer chromatography, and were identified by liquid chromatography–mass spectrometry, liquid chromatography–nuclear magnetic resonance and/or co-chromatography with authentic reference substances.

The metabolite pattern in urine, faeces and bile was complex but qualitatively independent of the sex and the dose level, with some quantitative variations. The following 11 metabolites were isolated:

- 3″-*O*-desmethyl-avermectin B_{1a} (3″DM[1]);
- 24a-hydroxy-avermectin B_{1a} (24aOH);
- 27-hydroxy-avermectin B_{1a} (27OH);
- 28-hydroxy-avermectin B_{1a} (28OH);
- 3″-*O*-desmethyl, 24a-hydroxy-avermectin B_{1a} (3″DM,24aOH);
- 3″-*O*-desmethyl, 27-hydroxy-avermectin B_{1a} (3″DM,27OH);
- 3″-*O*-desmethyl, 4a-hydroxy-avermectin B_{1a} (3″DM,4aOH);
- desoleandrosyl, 3′-*O*-desmethyl, 4a-hydroxy-avermectin B_{1a} (DO,3′DM,4aOH);
- 8a-oxo-avermectin B_{1a} (8aOxo) (code: NOA 448111);
- 8a-hydroxy-avermectin B_{1a} (8aOH) (code: NOA 448112);
- 4-hydroxy, 8a-oxo-avermectin B_{1a} (4OH,8aOxo) (code: NOA 457465).

Unchanged avermectin B_{1a} and the metabolites 3″DM, 24aOH, 27OH, 3″DM,24aOH and 3″DM,27OH represented the majority of the faecal radioactivity. Avermectin B_{1a} accounted for about 25–45% of the dose, and the individual metabolites 3″DM, 24aOH, 27OH, 3″DM,24aOH and 3″DM,27OH accounted for 3–27%. The minor metabolites 28OH, 3″DM,4aOH and DO,3′DM,4aOH each accounted for 0.4–2%. The soil metabolites 8aOxo (NOA 448111), 8aOH (NOA 448112) and 4OH,8aOxo (NOA 457465) were additionally detected in faeces, accounting for 0.3%, 0.4% and 0.08% of the dose, respectively.

The major faecal components, avermectin B_{1a} and the metabolite 3″DM, were not present in urine. The metabolites 24aOH, 27OH, 3″DM,24aOH and 3″DM,27OH represented the majority of the urinary radioactivity, each accounting for 0.02–0.4% of the dose. The metabolites 28OH and DO,3′DM,4aOH were detected in very small quantities, each less than 2% of the dose.

In bile, the major components detected were 3″DM,24aOH, 3″DM,27OH, 3″DM and avermectin B_{1a}, each accounting for 0.1–1.2% of the dose. The metabolites 27OH and DO,3′DM,4aOH were detected in very small quantities, less than 0.1% and less than 0.3% of the dose, respectively. Avermectin B_{1a} was the major component detected in the fat and muscle, accounting for 92% and 72% of fat or muscle radioactivity, respectively. Metabolite 3″DM accounted for 1.7% and 19% of the dose in the fat and muscle, respectively.

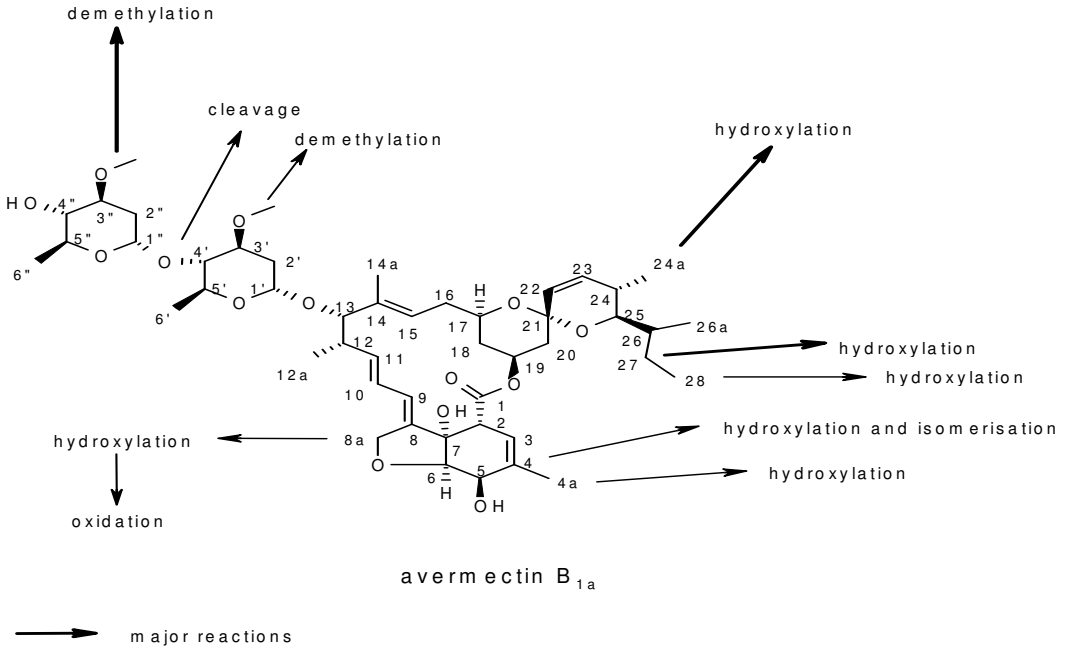
Based on the structures identified, the metabolism of avermectin B_{1a} in the rat proceeds predominantly via demethylation, hydroxylation, cleavage of the oleandrosyl ring and oxidation reactions (Fig. 4). The metabolites identified are the result of the following reaction steps:

- demethylation at the oleandrosyl ring, as in 3″DM;
- hydroxylation at different positions, as in 24aOH, 27OH, 28OH and 8aOH (NOA 448112); combined with demethylation, as in 3″DM,24aOH, 3″DM,27OH and 3″DM,4aOH;
- cleavage of the oleandrosyl ring, as in DO,3′DM,4aOH;
- oxidation of the 8a position, as in 8aOxo (NOA 448111) and 4OH,8aOxo (NOA 457465).

The metabolic pathways of avermectin B_{1a} in the rat are proposed in Fig. 5.

[1] Abbreviated from 3″-DM-B_{1a}, as shown in Fig. 3 and as used in the description of the study by Maynard, Wislocki & Lu (1986) above.

Fig. 4. Proposed reaction scheme for the metabolism of avermectin B_{1a} in rats



Source: Briswalter (2003b)

In another study (Briswalter, 2003a), the metabolite pattern in urine and faeces was complex but qualitatively independent of the sex and the dose level, with some quantitative variations. The metabolite pattern of avermectin B_{1b} was qualitatively similar to that obtained with avermectin B_{1a}, with some minor variations.

Unchanged avermectin B_{1b} accounted for 9–17% of the dose. The major metabolite fractions detected in excreta were fractions B7, B10 and B13, each accounting for 7–33% of the dose. It is considered that these fractions could correspond to fractions A7, A10 and A13 after administration of avermectin $B_{1a.}$ These metabolite fractions, identified as 3″DM,24aOH, 3″DM and 24aOH, respectively, were the major metabolites identified in the course of the metabolism study of avermectin B_{1a} in the rat.

Therefore, it can be concluded that the metabolic pathways of avermectin B_{1a} and avermectin B_{1b} in the rat are similar.

2. Toxicological studies

2.1 Acute toxicity

Studies of the acute toxicity of abamectin are summarized in Table 7, and acute dermal and eye irritation and skin sensitization studies with abamectin are summarized in Table 8.

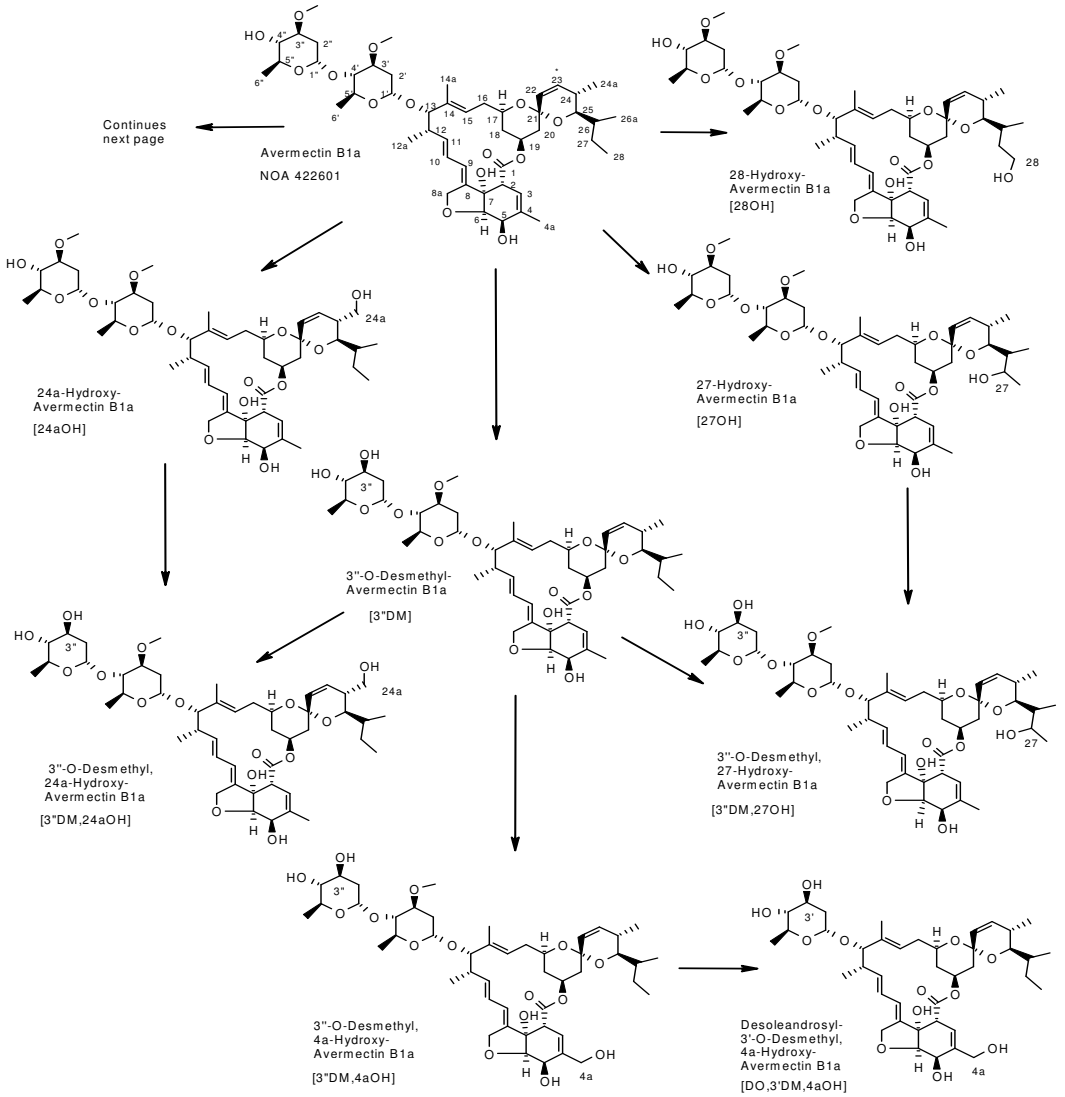
2.2 Short-term studies of toxicity

(a) Oral administration

Rats

In a dose range–finding study, groups of 10 male and 10 female rats (CRCD strain) approximately 3 weeks of age (weighing 37–59 g) were treated orally with abamectin technical (MK-0936, batch no. L-676,863-00V50, purity 94.0%) for 8 weeks by admixture in the diet at a constant

Fig. 5. Proposed pathways for the metabolism of avermectin B_{1a} in rats



Source: Briswalter (2003b)

concentration of 0, 5, 20, 40 or 60 ppm. The 20 ppm dietary concentration was increased to 25 ppm at the start of week 7. Severe toxicity and excessive mortality in the groups treated at 60 and 40 ppm necessitated termination of the surviving animals on days 5 and 15, respectively. Two additional groups of animals, treated at 10 and 15 ppm, were placed on test in week 5 and were treated for 4 weeks only. Mean achieved dose levels were 0.5, 1.2, 1.9, 2.2 and 5.0 mg/kg bw per day for 5, 10, 15, 20/25 and 40 ppm, respectively. A dose range could not be calculated for the 60 ppm group. Samples of all diets prepared in weeks 3 and 7 were analysed for test substance concentration. Further diets were prepared, sampled and analysed for homogeneity and stability. The animals were observed daily for mortality and clinical signs of toxicity, and feed consumption was measured for a 6-day period each week. Body weights were recorded pretest and weekly thereafter. All survivors were killed and discarded without necropsy after 4 weeks (10 and 15 ppm) or 8 weeks (0, 5 and 20/25 ppm) of treatment.

Table 7. Summary of acute toxicity studies with abamectin

Species	Strain	Sex	Route	Purity (%)	LD_{50}/LC_{50}	Reference
Rat	Crl:CD[SD] BR	M + F	Oral[a]	89.3	M: 232 mg/kg bw[b] F: 214 mg/kg bw Combined: 221 mg/kg bw	Glaza (2001)
Rat	CRCD	M + F	Oral[c]	91.4	M: 8.7 mg/kg bw F: 12.8 mg/kg bw[d]	Robertson (1981a)
Rat	RccHan:WIST	M + F	Dermal	89.3	M: > 2 000 mg/kg bw F: > 700 and < 2 000 mg/kg bw	Sieber (2011)
Rabbit	New Zealand White	M + F	Dermal	94	> 2 000 mg/kg bw	Gordon (1984a)
Rat	Crl:HanWis G1x:BRL) BR	M + F	Inhalation (4 h)	96.7	< 0.21 mg/L (MMAD 4.2 µm)	Ruddock (2001a)
Rat	Alpk:AP_fSD	M + F	Inhalation (4 h)	88.3	> 0.034 and < 0.21 mg/L[e] (MMAD: 2.11–2.29 µm)	Noakes (2003)

bw: body weight; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; MMAD: mass median aerodynamic diameter

[a] Vehicle: water.

[b] Clinical signs of toxicity for both males and females at a dose of 275 mg/kg bw included hypoactivity, staggered gait, dyspnoea, red stained face, wet area around the mouth, mydriasis, lacrimation, wet urogenital area, thin appearance, hunched posture, cold to the touch, prostration and tremors. The three surviving animals had fully recovered by day 8. All surviving animals of this group exhibited body weight gain throughout the study. Clinical signs of toxicity in both males and females in the 500 mg/kg bw dose group included hypoactivity, staggered gait, wet/brown area around the mouth, lacrimation, possible bronchial congestion, thin appearance, hunched posture, hypersensitivity, dyspnoea, cold to the touch, prostration, tonic convulsions and tremors. The surviving female returned to a normal appearance by day 6 after treatment and exhibited body weight gain during the study. At necropsy, the findings consisted of ocular, nasal and/or oral discharges of variable consistency and perianal/perineum staining. All other findings were considered incidental and not test material related. There were no visible lesions in any of the animals that survived to study termination.

[c] Vehicle: sesame oil.

[d] Clinical signs of toxicity comprising ataxia and whole-body tremors occurred in all dose groups (6.67, 10, 15, 22.5 and 33.75 mg/kg bw) within 3 hours of treatment. The symptoms persisted until day 6 in all male groups, but females treated at 6.67 mg/kg bw were essentially of normal appearance on day 2. Females treated at higher dose levels continued to show symptoms until day 4. Deaths occurred in all groups except for females treated at 6.67 mg/kg bw. Death occurred from 3 hours to 6 days and from 5 hours to 5 days post-treatment in males and females, respectively. The body weight gain of survivors was unaffected by treatment at all dose levels. There were no treatment-related gross lesions at necropsy, and all gross and histological lesions were considered to be incidental to treatment.

[e] Clinical signs characteristic of mild respiratory tract irritation were seen immediately after day 1 for all animals exposed to 0.051 mg/L and for four females at 0.034 mg/L. Changes indicative of mild to moderate systemic toxicity were also apparent following exposure at 0.051 mg/L, although a full recovery from all clinical signs was apparent for all surviving animals by day 15.

Table 8. Summary of acute dermal and eye irritation and skin sensitization studies with abamectin

Species	Strain	Sex	End-points	Purity (%)	Result	Reference
Rabbit	New Zealand White	M + F	Skin irritation	94	Not irritating	Robertson (1981b)
Rabbit	New Zealand White	M + F	Eye irritation	89.3	Not irritating	Glaza (2000)
Guinea-pig	Crl:[HA]BR	F	Skin sensitization	96.7	Not sensitizing (M&K)	Ruddock (2001b)

F: female; M: male; M&K: Magnusson and Kligman

Sixteen animals treated at 60 ppm died on day 3 or 4 of the test, and the remaining animals were terminated on day 5. Ten animals treated at 40 ppm died on days 3–5, and the remaining animals were terminated on day 15. One female rat treated at 15 ppm was killed in a moribund condition on day 16. As the animal had an enlarged and misshapen cranium, its condition is considered to be due to a congenital abnormality, hydrocephalus, rather than to treatment with abamectin. All other animals survived the designated treatment periods. Treatment-related clinical signs comprising tremors, decreased activity, red-coloured discharge in the oronasal region and staining of the urogenital area first occurred on day 2 in animals treated at 40 and 60 ppm. Slight tremors and/or urogenital staining also occurred in some animals treated at 15 and 20 ppm. Tremors persisted in some animals treated at 40 ppm until sacrifice on day 15, but animals treated at 15 and 20 ppm showed improving conditions after week 1 and, with the exception of some animals with unkempt fur and urogenital area staining, were of normal appearance. Treatment-related clinical signs at 15 and 20 ppm were no longer apparent after week 3. Animals treated at 5 and 10 ppm did not show clinical signs of toxicity, other than one female at 5 ppm that displayed slight tremors on day 2 only. As tremors did not occur at 10 ppm, the transient slight tremors in one female at 5 ppm are considered to be of equivocal toxicological significance.

Treatment at 40 ppm elicited an approximate 52% reduction in body weight gain in both sexes during the 2 weeks of treatment prior to sacrifice. Treatment at 15 and 20/25 ppm elicited transient growth retardation, 9.4–17.7% and 15.2–18.7%, respectively, in both sexes during the first 2 weeks of treatment only. Body weight gain was unaffected by treatment at 5 and 10 ppm. The feed consumption of both sexes treated at 40 ppm was reduced by 24.9–47.0% during the 2 weeks of treatment prior to sacrifice, but was unaffected by treatment at dietary concentrations up to 20/25 ppm. No gross or histological investigations were performed.

In view of the above, the NOAEL in rats of both sexes was 10 ppm abamectin technical (equivalent to 1.2 mg/kg bw per day), based on the occurrence of typical clinical signs and reduction in body weight gain at 15 ppm (equivalent to 1.9 mg/kg bw per day) and higher. This represented the NOAEL after 4 weeks of dosing; however, based on the lack of progression of changes observed at 20/25 ppm (equivalent to 2.2 mg/kg bw per day) between weeks 4 and 8, this NOAEL is also considered to be applicable after 8 weeks.

The study was not GLP compliant (Gordon, 1984b).

Dogs

In a 12-week dose range–finding study, five groups of two male and two female Beagle dogs (30–31 weeks old, weighing 7.8–12.0 kg) were treated continuously for 12 weeks with abamectin technical (MK-0936, batch no. L-676,863-00V50, purity 94%) by admixture in the diet at variable concentrations designed to provide dose levels of 0, 0.25, 0.5, 1.0 and 4.0 mg/kg bw per day. Treatment of the highest-dose group was discontinued for 9 days on day 20 and reinstated at a dose level of 2.0 mg/kg bw per day from day 29. Owing to persistent adverse reactions to treatment, the group was killed and discarded without necropsy on day 36. All animals were examined 7 days/week, with less detailed observations recorded on weekends and holidays. Body weights were recorded twice a week, and feed consumption was measured daily. A qualitative evaluation of the pupil response to light was performed daily on weekdays. Necropsy and postmortem examinations were not performed at the end of the study.

No deaths occurred at any dose level, but the animals treated at 4.0/2.0 mg/kg bw per day were killed and discarded on day 36. During treatment at 4.0 mg/kg bw per day, feed consumption was markedly reduced, generally to less than 25% of the ration offered. When the dose level was reduced to 2.0 mg/kg bw per day, feed consumption remained reduced by approximately 50%. In one female dog, rapid respiration, disorientation, tremors, weakness and slight uncoordination occurred on 1–3 occasions during treatment at 4.0 mg/kg bw per day. These signs disappeared on suspension of dosing and did not reappear on commencement of treatment at 2.0 mg/kg bw per day. Prior to sacrifice, the animals either lost weight or did not gain weight.

Absence of pupil constriction in response to light (i.e. mydriasis) occurred, starting in week 1, in the animals treated at 1.0 and 4.0 mg/kg bw per day. The effect also occurred in the latter group when the dose level was reduced to 2.0 mg/kg bw per day. The observation was not consistent but occurred 1–5 times/week in individual animals. Absence of pupil constriction did not occur in animals treated at 0.25 or 0.5 mg/kg bw per day.

The body weight gains of the groups treated at 0.25, 0.5 and 1.0 mg/kg bw per day were unaffected by treatment. The feed consumption of these groups was not reported.

A very steep dose–response curve is evident for orally administered abamectin in the dog, and the maximum tolerated dose (MTD) was clearly exceeded at dose levels of 2.0 mg/kg bw per day and higher. Therefore, the MTD is 1.0 mg/kg bw per day. The NOAEL was identified as 0.5 mg/kg bw per day, based on the occurrence of mydriasis starting in week 1 of treatment at 1.0 mg/kg bw per day. Markedly reduced feed consumption, body weight loss and clinical signs of intoxication were observed at 2.0 mg/kg bw per day and above.

The study was not GLP compliant (Gordon, 1984c).

In an 18-week study, four groups of three male and three female Beagle dogs (26–42 weeks old, weighing 7.5–12.5 kg) were treated daily with avermectin B_{1a} (batch nos 9603-10-13-1, P14 and P17, purities not specified) by oral gavage as a solution in sesame oil at a dose of 0.25, 0.5, 2.0 or 8.0 mg/kg bw per day. Another two groups of dogs, treated with either tap water or sesame oil, served as control groups.

Treatment of the groups with 8.0 and 2.0 mg/kg bw per day was discontinued after one and three doses, respectively, and the survivors were maintained untreated until study termination. All animals were examined 5 days/week, with less detailed observations recorded on weekends and holidays. Body weights were recorded twice a week, and rectal temperatures were recorded daily from day 87. Ophthalmological examinations were performed pretest and in weeks 3, 8, 11 and 16. A qualitative evaluation of the pupil response to light was performed daily for the first 3 weeks and 5 days/week thereafter. Haematology and serum biochemistry determinations were performed on all animals (fasted) pretest and in weeks 1 (8.0 mg/kg bw per day only), 4, 8, 13 and 17. Additional haematological investigations were performed in weeks 13 and 15 on one animal treated at 0.25 mg/kg bw per day. Urine analysis was performed in weeks 4, 8, 13 and 18.

Electrocardiographic (ECG) recordings were performed on all animals pretest and in weeks 2, 3, 8, 12 and 17. ECG recordings were also performed on day 1 for animals treated at 8.0 mg/kg bw per day. All animals, decedents and fasted survivors, were examined postmortem, and tissues were preserved. Organ weights were recorded for animals surviving to termination. Major organs and tissues and all gross lesions from animals in all treated and control groups were processed to sections stained with haematoxylin and eosin and examined microscopically. Frozen liver samples were also stained with Oil Red O to detect the presence of neutral lipid. Frozen kidney samples from the 0.25 mg/kg bw per day group and the sesame oil control group were stained with Oil Red O.

There were no deaths or treatment-related clinical signs in dogs treated at 0.25 mg/kg bw per day. One dog died in the 0.5 mg/kg bw per day dose group after 18 doses. Three dogs in the 2.0 mg/kg bw per day group died after the third dose, and three dogs died within 24 hours of receiving the first dose in the 8.0 mg/kg bw per day group. Signs of toxicity at 0.5, 2.0 and 8.0 mg/kg bw per day included whole-body muscular tremors, ataxia, mydriasis and ptyalism. In addition, at 2.0 and 8.0 mg/kg bw per day, tonic convulsions and emesis were observed. Dosing of the 2.0 and 8.0 mg/kg bw per day groups was discontinued after the third and first doses, respectively, owing to the severity of clinical signs.

Body weight gain was unaffected by treatment at 0.25 mg/kg bw per day, but body weight loss occurred in some animals treated at 0.5 or 2.0 mg/kg bw per day. Survivors at the higher dose levels showed weight gains comparable with those of the controls after the cessation of treatment, with the exception of one animal treated at 8.0 mg/kg bw per day. There were no treatment-related

changes in haematology and serum chemistry profiles at dose levels up to 2.0 mg/kg bw per day. Treatment-related effects at 8.0 mg/kg bw per day were confined to the samples taken 4 hours after the first dose.

Red and white blood cell counts, haemoglobin concentrations and haematocrit values were slightly elevated 4 hours after the first dose at 8.0 mg/kg bw per day. Serum glucose concentrations at 8.0 mg/kg bw per day were elevated in four animals, and protein levels were slightly raised in two animals, but these are considered to be sequelae of stress. Urine analysis profiles were unaffected by treatment at all dose levels. No treatment-related ocular defects were apparent at any dose level. The pattern of ECG traces was unaffected by treatment up to 2.0 mg/kg bw per day. In week 1, animals treated at 8.0 mg/kg bw per day showed bradycardia (range 32–109 beats/minute) and an increased QT interval (range 0.24–0.28 second). The effects were greatest in the animals that subsequently died. Subsequent ECG traces from all dose groups showed no abnormalities. Individual ECG data were not presented in the report.

An addendum to this study on supplemental histology was provided. Treatment-related histopathological lesions were confined to decedent animals at all dose levels. Consistent changes were confined to the hepatobiliary system in all but one decedent. Diffuse vacuolation of hepatocytes occurred in three, two and one animal treated at 8.0, 2.0 and 0.5 mg/kg bw per day, respectively. Oil Red O staining did not reveal neutral lipid in the vacuoles. Minimal to slight oedema of the gallbladder, primarily in the subserosal connective tissue, occurred in two animals treated at 2.0 mg/kg bw per day and two animals at 8.0 mg/kg bw per day. The female treated at 2.0 mg/kg bw per day that died on day 49 following perforation of the jejunum and peritonitis had severe, multifocal necrotizing enteritis. As no other animals showed gastrointestinal lesions, either gross or microscopic, it is considered that the lesion was not treatment related. There were no treatment-related organ weight changes or histopathological alterations in surviving animals at any dose level.

The above information suggests a very steep dose–response curve for orally administered avermectin B_{1a} in the dog, and the MTD was clearly exceeded at dose levels above 0.5 mg/kg bw per day. A NOAEL of 0.25 mg/kg bw per day was identified in both sexes, based on mortality, clinical signs of toxicity, reduced body weight gain or body weight loss, and histopathological changes in the liver at 0.5 mg/kg bw per day. Signs of toxicity were observed starting on day 7 at 0.5 mg/kg bw per day, on day 3 at 2.0 mg/kg bw per day and on day 1 (within 3 hours after dosing) at 8.0 mg/kg bw per day.

The study was not GLP compliant. However, the supplemental histology study was GLP compliant (Allen & Robertson, 1976; Allen, 1982).

In a 53-week dietary study, four groups of six male and six female Beagle dogs (20–24 weeks old, body weight range 4.9–8.7 kg) were treated orally with abamectin technical (MK-0936, batch no. L- 676,863-00V54, purity > 89% by liquid chromatography) by dietary admixture at variable concentrations calculated to provide dose levels of 0, 0.25, 0.5 and 1.0 mg/kg bw per day. The dose levels were selected on the basis of a 12-week study in dogs in which 0.5 mg/kg bw per day was found to be the NOAEL (Gordon, 1984c). Clinical signs were recorded daily, and the eyes were checked for direct light-induced pupil constriction on weekdays. Body weights were recorded weekly, and feed consumption was usually measured 4 times/week. Opthalmoscopic examinations were performed pretest and in weeks 12, 25, 40 and 52. Haematology, serum biochemistry and urine analyses were performed pretest and in weeks 4, 8, 12, 26, 38 and 52. All animals, decedents and survivors, were subjected to necropsy, postmortem examination, organ weight recording (not decedents) and extensive tissue preservation.

All tissues from all animals were processed to sections stained with haematoxylin and eosin and examined microscopically without knowledge of the treatment group. Frozen sections of liver and kidney were stained with Oil Red O to indicate the presence of neutral lipids.

Deaths were confined to the highest dose level, 1.0 mg/kg bw per day. Two male animals were killed in a moribund condition, one in week 33 and another in week 38. One animal showed

Table 9. Incidence of absent or decreased pupil reactivity to direct light

	0 mg/kg bw per day	**0.25 mg/kg bw per day**	**0.5 mg/kg bw per day**	**1.0 mg/kg bw per day**
No. of observations of absent or decreased pupil reactivity to direct light	0	4	101 (3%)	422 (15%)
No. of dogs in which observation seen	–	Two times in each of two animals	In all but one animal	At least once in all animals treated

bw: body weight
Source: Gordon (1984d)

weight loss and lethargy, and the other showed excessive salivation, unsteadiness and lateral recumbence prior to sacrifice. A third male dog died in week 38 without clinical signs of toxicity. The feed consumption pattern of this animal indicated an ingested dose of 1.3–1.5 mg/kg bw per day for several days before death. Treatment-related clinical signs in surviving animals at 1.0 mg/kg bw per day were confined to absent or decreased pupil reactivity to direct light (Table 9). The observation was made 422 times at 1.0 mg/kg bw per day (occurrence rate approximately 15%) and 101 times at 0.5 mg/kg bw per day (occurrence rate approximately 3%). It also occurred 4 times at 0.25 mg/kg bw per day (twice in each of two animals). The effect occurred at least once in all animals treated at 1.0 mg/kg bw per day and in all but one animal at 0.5 mg/kg bw per day, but the presence of the effect in individuals was inconsistent. As the observation is highly subjective, was not performed blind and occurred inconsistently at 0.5 mg/kg bw per day, the significance of the very low incidence at 0.25 mg/kg bw per day is considered equivocal.

Body weight gain and feed consumption were unaffected by treatment at 0.25 and 0.5 mg/kg bw per day. The overall mean body weight gain of the group treated at 1.0 mg/kg bw per day was 29.1% lower than that of concurrent controls, and feed consumption was markedly reduced. Concentrations of abamectin technical in diet in the high-dose group appeared to be unpalatable. There were no treatment-related ophthalmoscopic findings at any dose level.

There were no haematological changes at any dose level in surviving animals. However, one male treated at 1.0 mg/kg bw per day and killed in week 38 showed slight leukocytosis, predominantly neutrophilia, and slightly elevated packed cell volume just prior to sacrifice. Treatment-related serum biochemistry effects occurred at 1.0 mg/kg bw per day only. Serum urea and total protein concentrations were slightly reduced throughout the treatment period. The changes in serum biochemistry are considered to be physiological sequelae to markedly reduced feed consumption. No effects on urine analysis profiles occurred at any dose level. There were no treatment-related organ weight changes or gross or histopathological alterations at any dose level in all animals, including three male decedents treated at 1.0 mg/kg bw per day.

A NOAEL for abamectin technical of 0.25 mg/kg bw per day was identified in the dog, based on mydriasis at 0.5 mg/kg bw per day. Signs of toxicity were observed starting in the first week at 0.5 and 1.0 mg/kg bw per day.

The study was GLP compliant (Gordon, 1984d).

Clinical signs of neurotoxicity in dogs, including their time of first appearance, in the above three studies are compared in Table 10.

Table 10. Clinical signs observed in dog studies

Study description	0.25 mg/kg bw per day	0.5 mg/kg bw per day	1.0 mg/kg bw per day	2.0 mg/kg bw per day	4.0 mg/kg bw per day	8.0 mg/kg bw per day
12-week dose range–finding study (feeding) (0.25, 0.5, 1.0, 4.0/2.0 mg/kg bw per day) (2 M/2 F)	Mydriasis was never noted	Mydriasis was never noted	Mydriasis was noted starting in week 1, 1–5 times/week	Mydriasis was noted; feed consumption and body weight were markedly reduced; dogs were sacrificed on day 36	Mydriasis was noted starting in week 1, 1–5 times/week; tremor, rapid respiration and weakness were seen; dosing was ceased on day 20 (marked decrease in feed consumption and body weight) and reduced to 2.0 mg/kg bw per day on day 29	–
18-week toxicity study (gavage) (0.25, 0.5, 2.0 and 8.0 mg/kg bw per day) (3 M/3 F)	Mydriasis was never noted	Mydriasis was seen on treatment day 7 in one dog; in week 2, mydriasis was seen in 4/6 dogs; whole-body tremor, ataxia and ptyalism were seen in one dog at week 2 and persisted until the end of the study	–	Mydriasis, ataxia, ptyalism and anorexia were seen in all dogs by day 3 of dosing; after the third dose, three dogs died with signs of convulsion or tremors prior to death; dosing was ceased after the third dose	–	Ptyalism, emesis, ataxia and whole-body tremors were seen within 3 h after first dose, and three dogs died after first dose; mydriasis was seen 24 h after dosing in surviving dogs; dosing was ceased after the first dose
53-week toxicity study (feeding) (0.25, 0.5 and 1.0 mg/kg bw per day) (6 M/6 F)	Mydriasis was noted in week 10 in two dogs, but not before or after that time; not treatment related	Mydriasis was noted in week 1 in one dog (1×); by week 6, five dogs had mydriasis	Mydriasis was noted in week 1 in one dog (2×); by week 3, four dogs had mydriasis; by week 11, 11 dogs had mydriasis; by week 38, three males died	–	–	–

bw: body weight; F: females; M: males
Source: Allen & Robertson (1976); Allen (1982); Gordon (1984c,d)

(b) Exposure by inhalation

Rats

In a 5-day preliminary inhalation toxicity study, groups of two male and two female HsdBrlHan: WIST rats were exposed nose-only to abamectin technical (milled) at 0 (control), 1, 4, 10 or 25 µg/L for a 6-hour period daily, for up to 5 consecutive days. Clinical observations were made daily throughout the study. Body weights were measured daily, and feed consumption was measured continuously throughout the study. At the end of the scheduled period, the animals were killed and subjected to a full postmortem examination. Selected organs were weighed, and specified tissues were taken for subsequent histopathological examination. Abamectin technical is non-volatile (vapour pressure $< 3.7 \times 10^{-6}$ Pa), and it exists in physical form as a solid "wet cake".

The test atmospheres generated (1, 4, 10 and 25 µg/L) were acceptable with regard to stability and physical characteristics.

At 25 µg/L, two females were killed on day 2 (one before the exposure period on that day, the other after 4.5 hours of exposure), and one male was killed on day 3 (before the exposure period on that day), all as a result of adverse clinical signs (including reduced splay reflex and stability, tremors, shaking, irregular breathing, decreased visual placement, tail erection, hunched posture, piloerection, decreased activity, stains around the mouth and nose), body weight loss and markedly reduced feed consumption. There were no further exposures at this concentration. The surviving male was allowed to recover between days 3 and 6. At 10 µg/L, one female was killed after 2 consecutive exposure days as a result of adverse clinical signs, body weight loss and reduced feed consumption. The other female lost weight, had reduced feed consumption and exhibited some adverse clinical signs. Males at this level showed some clinical signs, but had normal growth and feed consumption. The three remaining animals in this group continued to be exposed as scheduled until termination. Concentrations of 25 and 10 µg/L were, as a result of the findings, considered to be too high for a subsequent repeated-exposure study. At 4 µg/L, reduced splay reflex was seen in one female between days 4 and 6 and in one male on day 5. At 1 µg/L, growth of males was slightly lower than in controls, and one female had reduced splay reflex between days 5 and 6. There were no treatment-related microscopic findings, at any of the dose levels tested, in animals exposed for 5 consecutive days.

In view of the above findings, no exposure concentrations higher than 4 µg/L were recommended for the subsequent 30-day inhalation study.

The study was GLP compliant, and a QA statement was attached (Pinto, 2006a).

In the main study, groups of 10 male and 10 female Han Wistar (HsdBrlHan:WIST) rats were exposed nose-only for 6 hours/day to a target abamectin technical (milled) concentration of 0 (control), 0.1, 0.5 or 2.5 µg/L for 5 days/week over a 30-day study period.

Detailed clinical observations were made weekly. Body weights were measured weekly, and feed consumption was measured continuously throughout the study. Eyes were examined pre-study and pre-termination. A functional observational battery and an assessment of locomotor activity were performed in week 4. On day 30, the surviving animals were killed and fully examined postmortem. Cardiac blood samples were taken for clinical pathology from all animals, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

The test atmospheres generated were acceptable with regard to general stability and physical characteristics. Concentrations were close to target concentrations, and particle sizes were within the required range.

One female exposed to 2.5 µg/L was found prostrate, shaking and gasping with a swollen head on day 2, prior to the start of the exposure period. This animal was sent for necropsy. One female exposed to 2.5 µg/L was ungroomed, with stains around the mouth, hunched posture and piloerection on day 15 (week 3) only. Two females at this exposure concentration had abnormal respiratory noise in week 5.

Body weight for females exposed to 2.5 µg/L was lower than control values on days 15 and 22 (maximum difference 5%). This difference was predominantly due to one female that had poor growth during this period.

Locomotor activity was statistically significantly lower than in controls for females exposed to 2.5 µg/L, both overall (1–50 minutes) and during minutes 36–40. Activity was generally lower during the latter part of the assessment period for these animals.

Plasma alanine aminotransferase activity was statistically significantly lower than in controls for males exposed to 0.5 and 2.5 µg/L. Spleen weight was statistically significantly higher than control values for males exposed to 2.5 µg/L. Owing to the small magnitude of the changes and in the absence of any histopathology, these findings are considered to be of no toxicological significance. There were no treatment-related macroscopic or microscopic findings at any exposure concentration. No abnormalities were seen in males at any exposure concentration or in females exposed to 0.1 or 0.5 µg/L.

The study was GLP compliant, and a QA statement was attached (Pinto, 2006b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 74 male and 74 female 6-week-old mice (Crl:CD-1 (ICR)BR strain, body weight range 15.5–33.2 g) were administered abamectin (MK-0936, batch no. L-676,863-00V54, initial purity 91.1%, purity determined to be 89.0% weight per weight [w/w] in week 34) orally for 93 weeks, by admixture in the diet, at concentrations adjusted to provide dose levels of 0 (control I), 0 (control II), 2.0, 4.0 and 8.0 mg/kg bw per day. All female groups started on study were killed and discarded shortly after the initiation of treatment because of the presence of tremors at all dose levels within 24 hours and deaths at 4.0 and 8.0 mg/kg bw per day. New groups of females were started on test, at the same dose levels, approximately 1 month later. Treatment of the male group with 8.0 mg/kg bw per day was discontinued after 89 weeks when survival had reached 40%. The stability and homogeneity of abamectin in the diet were confirmed prior to initiation of treatment, and achieved dietary concentrations were assayed for each dose group on 6- to 8-week pooled samples throughout the study.

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

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

Treatment-related tremors occurred in several females from all the original treatment groups after 1 day. Seven and three females treated at 8.0 and 4.0 mg/kg bw per day, respectively, died on day 2. Treatment was withdrawn, the affected animals were replaced and treatment was recommenced

1 week later. Treatment-related tremors recurred in some females from all groups. Therefore, all female mice were terminated, and new groups of females were restarted on study 4 weeks later. Following restart of the study, only two females at 8.0 mg/kg bw per day showed sporadic tremors, one in week 89 and the other in week 91. Treatment-related tremors did not occur at lower dose levels, although one female treated at 4.0 mg/kg bw per day showed circling and tremors 1 day prior to death in week 49. As the animal was subsequently diagnosed with metastatic lymphoma of the pituitary gland, the tremors are considered to be secondary to the tumour. Treatment-related tremors did not occur in males at any dose level. Transient tremors, considered not to be treatment related, occurred 1 day before sacrifice, in a moribund control II male and two moribund males treated at 8.0 mg/kg bw per day.

A treatment-related increase in mortality occurred in males treated at 8.0 mg/kg bw per day ($P = 0.012$), and treatment of the group was discontinued after 89 weeks. There was no effect on male survival at lower dose levels or in any of the female groups ($P > 0.05$). Male mortality incidences after 93 weeks of treatment were 48%, 48% and 68%, in order of ascending dose level, compared with an incidence of 50% in both male control groups. Female mortality incidences after 93 weeks of treatment were 40%, 40% and 32%, in order of ascending dose level, compared with incidences of 28% and 32% in female control groups. Treatment-related effects on weight gain were confined to the female group treated at 8.0 mg/kg bw per day. At this dose level, there was an overall reduced weight gain of 21.4% compared with the mean weight gain of the control groups. There was no effect on feed consumption at any dose level. No treatment-related ocular defects occurred, and there were no treatment-related changes in the haematological and serum biochemistry profiles at any dose level. Several individual animals in all treated and control groups showed isolated, abnormal haematological and serum biochemistry parameters; in general, these could be related to specific clinical observations or are common geriatric or morbid changes in mice.

At necropsy, there were no treatment-related effects either on organ weights or on the nature and distribution of gross lesions. Statistical analysis of the incidence of tumour-bearing animals for each tumour site showed that there were no statistically significant positive or negative trends in incidence for any of the tumour sites in either sex. There were no treatment-related non-neoplastic histopathological findings at any dose level in either sex. An increased incidence of dermatitis occurred in males treated at 4.0 and 8.0 mg/kg bw per day. There were 3/49, 6/50 and 11/50 occurrences in male groups, in order of ascending dose level, compared with control incidences of 2/50 and 3/50. The increased incidences at 4.0 and 8.0 mg/kg bw per day are unlikely to be treatment induced, as dermatitis related to fighting among male cage-mates is common. The incidences of dermatitis in female groups were 0/50, 0/49 and 1/50, compared with control female incidences of 0/50 and 1/50. All other histopathological findings were evenly distributed between the control and treated groups and were of the type and severity that generally occur in the strain of mouse used.

In view of the above, the NOAEL for abamectin technical in mice was identified as 4.0 mg/kg bw per day in both sexes, based on increased mortality in males and reduced body weight gain in females at 8.0 mg/kg bw per day. There was no increase in tumour incidence in mice in this study.

The study was GLP compliant, and a QA statement was attached (Gordon, 1985a).

Rats

Groups of 65 male and 65 female 5-week-old Sprague-Dawley-derived rats (Crl:CD(SD)BR strain, body weight range 93–191 g) were administered abamectin technical (batch no. L-676,863-00V54, initial purity 91.1%, purity determined to be 89% w/w in week 51) orally for 104 weeks, by admixture in the diet, at concentrations adjusted to provide dose levels of 0 (control I), 0 (control II), 0.75, 1.5 and 2.0 mg/kg bw per day. The highest dose level was increased to 2.5 mg/kg bw per day for weeks 11 and 12, followed by 1 day off-dose.

Fifteen animals of each sex per group were designated for interim sacrifice at week 53, and 50 animals of each sex per group were sacrificed after 104 weeks of treatment for the oncogenicity study. Mortality checks and clinical observations were made daily, and detailed physical

examinations, including palpation, weekly. Body weights were recorded pretest and weekly thereafter. Feed consumption was measured weekly in 12 animals of each sex per group for 5 or 6 days/week. The eyes of all animals were examined pretest and of control and high dose group animals in weeks 26, 52/53, 76 and 102/103. Haematological, serum chemistry and urine analysis investigations were performed at weeks 12, 25, 38 and 51 in 10 animals of each sex per group designated for sacrifice after 52 weeks, in week 78 on 10 animals of each sex per group from the main study animals, and pre-terminally in all survivors. All decedent and surviving animals were subjected to detailed necropsy and postmortem examination. Organ weights were recorded for all animals that survived to scheduled sacrifice. Tissue/organ samples from all animals sacrificed at 52 weeks, those that died or were killed during the study and all animals killed at the end of the study were preserved. Microscopic examination of tissues was performed on all control (I) and high-dose animals killed at 52 weeks, all decedents from all groups scheduled to be killed after 52 weeks, and all decedents and survivors from all groups scheduled to be killed after 104 weeks. Mortality and body weight data (104-week groups only) were statistically analysed by the Mantel-Haenszel trend test, and tumour incidences by the extended Mantel-Haenszel procedure implementing adjustments for variables.

Treatment-related clinical signs comprising whole-body tremors and unthrifty appearance were confined to the group treated at 2.0 mg/kg bw per day and, in all but one instance, first occurred in week 12 after the dose level was increased to 2.5 mg/kg bw per day. The clinical signs persisted intermittently until termination. Tremors were also noted in a further two females at 2.0 mg/kg bw per day and in one female at 1.5 mg/kg bw per day, starting late in the treatment period. These females were all small animals (< 300 g) and are likely to have received a disproportionately high dose of abamectin technical. Mortality incidence was unaffected by treatment at all doses of abamectin technical. At termination, mortality incidences were 52%, 52%, 62%, 42% and 68% in males and 62%, 48%, 68%, 66% and 64% in females, in order of ascending dose level. All incidences of mortality in abamectin-treated groups were not significantly different ($P > 0.05$) from the combined control group incidence. A treatment-related increase in weight gain occurred in both sexes of all treated groups, the effect being inversely related to dose level in the top three doses. Consistently higher weight gains occurred in males throughout the treatment period; at termination, the overall weight gains were significantly ($P < 0.05$) increased by 21%, 9% and 5%, in order of ascending dose level. In females, the effect was confined to the first 60 weeks of treatment, during which time the weight gains of the treated groups were significantly ($P < 0.05$) increased by 26%, 10% and 7%, in order of ascending dose level. Thereafter, the weight gains were lower than the control values; at termination, the overall weight gains of treated females were not significantly different from those of the controls.

The feed consumption of all abamectin-treated groups was unaffected by treatment at all dose levels. Ocular abnormalities observed in both high-dose and control animals during the study were of the type commonly observed in rats, and none was considered to be treatment related in origin. There were no treatment-related changes at any sampling interval in the haematological, serum biochemistry or urine analysis profiles at any dose level. Several individual animals in all study groups showed isolated, abnormal blood or urine parameters; in general, however, these could be related to specific clinical observations or are common geriatric or morbid changes in rats.

There were no treatment-related gross lesions at any dose level either in decedents or in survivors at necropsy after 52 or 104 weeks of treatment. There were no treatment-related changes at any dose level in absolute or relative organ weights after 52 and 104 weeks of treatment. There were no treatment-related histopathological changes, either neoplastic or non-neoplastic, at any dose level after 52 weeks of treatment, including three animals at 2.0 mg/kg bw per day sacrificed because of tremors. No neuromuscular changes were found to account for the clinical signs. There were no treatment-related histopathological changes, either neoplastic or non-neoplastic, at any dose level after 104 weeks of treatment. All tumour incidences in both sexes were not statistically significantly different from the control groups. All histopathological changes observed after 52 and 104 weeks of treatment with abamectin are considered to be common spontaneous findings in rats of the age and strain used.

A NOAEL of 1.5 mg/kg bw per day was identified, based on the occurrence of clinical signs of toxicity at 2.0 mg/kg bw per day in both sexes. There was no increase in tumour incidence in rats in this study.

The study was GLP compliant, and a QA statement was attached (Gordon, 1985b).

2.4 Genotoxicity

Abamectin was tested for genotoxicity in four in vitro assays and one in vivo assay. All the results were negative (Table 11).

Table 11. Overview of genotoxicity tests with abamectin

End-point	Test system	Concentrations/doses tested	Purity (%)	Results	Reference
In vitro					
Mammalian point mutations	Chinese hamster V-79 cells	3, 4, 5, 6 $\times 10^{-3}$ mmol/L (−S9) 3, 4, 4.5, 5 $\times$ 10^{-2} mmol/L (+S9)	94	Negative	Gordon (1983)
Microbial point mutations	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	0, 3, 10, 30, 100, 300, 1 000 µg/plate	94	Negative	Gordon (1986a)
Microbial point mutations	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2 uvrA	0, 312.5, 625, 1 250, 2 500, 5 000 µg/mL	89.3	Negative	Deparade (2001)
Chromosomal aberrations	Chinese hamster ovary cells	0, 10, 15, 20, 25, 30, 35 $\times$ 10^{-2} mmol/L (−S9) 0, 5, 10, 15, 20, 25 $\times$ 10^{-3} mmol/L (+S9)	94	Negative	Gordon (1986b)
In vivo					
Chromosomal aberrations	CD-1 strain mouse bone marrow	0, 1, 2, 4, 12 mg/kg bw and killed after 6, 24 or 48 h	94	Negative	Blazak (1983)

bw: body weight; S9: 9000 × *g* supernatant fraction from rat liver homogenate

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

Abamectin technical (MK-0936, batch no. L-676,863-00V50, purity not reported) was administered orally to two successive generations (P and F_{1b}) of male and female rats, by gavage, at a dose level of 0 (vehicle only), 0.05, 0.12 or 0.40 mg/kg bw per day. Two litters were produced by both the P generation (F_{1a} and F_{1b}) and the F_{1b} generation (F_{2a} and F_{2b}).

Initially, four groups of 30 male and 30 female rats (Crl:CD(SD) BR strain, approximately 6 weeks old, weight range 113–194 g) were continuously treated daily, by gavage, with abamectin technical for 68 days prior to mating, throughout cohabitation, gestation and lactation for F_{1a} and F_{1b} litters, and subsequently for a minimum 16-day maternal rest period (females) and a further 50 days after weaning of the last F_{1b} litter (males). Selected F_{1b} progeny were similarly treated, but from

weaning until approximately 30 days after the last F_{2b} litter had been weaned. The test substance was administered at 5 mL/kg bw as a solution in sesame oil, at a dose level of 0 (vehicle only), 0.05, 0.12 or 0.40 mg/kg bw per day for 259 days and at least 177 days for P generation males and females, respectively, and for a maximum of 273 days for selected F_{1b} progeny. Individual doses were recalculated weekly for both the P and F_{1b} generations, except for females during gestation and lactation, when treatment volumes were readjusted at 3-day intervals. Samples of abamectin formulations were analysed for test concentration at 5/6-week intervals throughout the study. Achieved concentrations were within acceptable limits (not less than 90% of the nominal concentration), with the exception of the low-dose solution on three occasions, when achieved concentrations were 65–80% of the nominal concentration.

Matings were performed within groups on a 1:1 basis for 14 days, during which time vaginal lavage was performed daily to check for mating (day 0 of gestation = day on which sperm were observed in the vagina). Females failing to mate were rehoused with a proven male for a further 7 days. Litters were examined as soon as possible after birth (day 1 postpartum), and viability and pup weights were recorded on days 1, 4, 7, 14 and 21 postpartum. Litters were culled to 10 pups (five of each sex, where possible) on day 4, by random selection. Maternal behaviour and pup behaviour were recorded on pup weighing days. F_{1a} litters were weaned, killed and discarded without further examination on day 21 postpartum. Following a maternal rest period of at least 16 days, the P generation was remated to produce the F_{1b} generation. P generation females not mating or not producing a litter were killed and discarded after reproductive status had been confirmed. All other P generation females were killed and discarded after weaning of the F_{1b} litters. P generation males were killed, and testes and epididymides were weighed and preserved in Bouin's fluid and then discarded. F_{1b} litters were weaned between days 26 and 30 postpartum, and one or two pups of each sex per litter (32 pups of each sex per group) were randomly selected for extended evaluation. On day 26/27, a further one pup per litter was randomly selected from 10 litters per group for full gross examination, organ weights and microscopic pathological evaluation. All other F_{1b} progeny were killed and discarded. The selected F_{1b} progeny for extended evaluation were mated twice to produce the F_{2a} and F_{2b} generations in a manner similar to that followed for the P generation. Five F_{2b} pups of each sex per group were randomly selected for full gross examination, organ weights and microscopic pathological evaluation, and 10 pups of each sex per group were killed and submitted for skeletal examination. All other F_{2b} pups were killed at weaning, the eyes retained in fixative, and the pups discarded. Thirty days after weaning of the last F_{2b} litter, 10 male and 25 female F_{1b} parental animals per group were randomly selected for necropsy, full gross examination, organ weights and histopathology. The remaining F_{1b} parental animals were killed and discarded, but testes and epididymides were weighed and preserved in Bouin's fluid from the males, and the eyes of both sexes were preserved. All pre-weaned pups found dead were given a gross external and visceral examination, and the carcasses were processed for skeletal examination. Weaned animals that died or were killed in a moribund condition were subjected to necropsy, and gross lesions were processed and examined histologically. Parental clinical signs were recorded daily and body weights were recorded weekly throughout the study and at termination, except for females during gestation and lactation, when body weights were recorded at 3-day intervals. Parental animals dying or killed in a moribund condition were subjected to a full necropsy, gross examination and microscopic evaluation of gross lesions.

Body weights were analysed statistically by Bartlett's test for homogeneity of variance and analysis of variance (ANOVA). Litter data and durations of cohabitation and gestation were analysed by the Kruskal-Wallis test. Where significant, the data were further analysed using Dunn's multiple comparison test. Fertility and mating parameters were analysed by the variance test for homogeneity of the binomial distribution.

P generation. No treatment-related deaths or clinical signs occurred at any dose level, although nine animals died during the study. One, two and two males died in the groups treated at 0, 0.05 and 0.12 mg/kg bw per day, respectively, after receiving 103–216 doses, and two control and two treated females at 0.05 mg/kg bw per day died after receiving 95–171 doses. The causes of death, established by histopathological examination, were diverse; as the distribution of mortality did not show a dose–response relationship, the deaths were considered incidental to treatment. There was a

treatment-related increase in weight gain of male rats treated at 0.40 mg/kg bw per day (Table 12). Thus, at termination, the group mean body weight was increased by 16.2%. A similar, but less marked and transient, effect occurred in males at 0.12 mg/kg bw per day (terminal group mean body weight increased by 5.4%). A similar effect occurred prior to mating in females treated at 0.12 and 0.40 mg/kg bw per day. At the end of the premating period, group mean body weights were increased by 11.2% and 25.8%, respectively. Although increased weight gains at 0.12 and 0.40 mg/kg bw per day are considered treatment related, they are not considered to be adverse effects of treatment. Overall body weight gains of maternal animals during the first and second gestation and lactation periods were unaffected by treatment at all dose levels.

No effects were seen on mating/fertility parameters during breeding for F_{1a} litters. However, during breeding for the F_{1b} generation, the number of high-dose males (0.40 mg/kg bw per day) mating was significantly lower ($P < 0.01$) than in controls. For males, the mean number of days in cohabitation was significantly increased ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively) for the low-, middle- and high-dose groups. The statistically significant differences for mating duration for the F_{1b} litters at 0.05 and 0.12 mg/kg bw per day are considered incidental to treatment, as the control group showed an unusually short mating time. Similarly, as observed in males, for 0.40 mg/kg bw per day females, the mean number of days in cohabitation prior to mating was significantly increased ($P < 0.05$). Significantly fewer ($P < 0.01$) 0.40 mg/kg bw per day group females mated compared with controls. Although no measurements of estrous cycling were performed, it has been speculated that this effect on mating may have resulted from estrous cycling irregularities that occurred for P female rats following production of the F_{1a} litters. The effect on fertility and fecundity of P animals of both sexes during breeding for F_{1b} litters was not reproducible in breeding for F_{1a}, F_{2a} and F_{2b} litters. Furthermore, no histopathological correlates were evident in the reproductive organs of either sex of the P generation. Therefore, this is considered to be an isolated finding that is not related to treatment.

There were no treatment-related effects on the incidence of stillborn pups. However, the mortality of neonate rats of F_{1a} and F_{1b} litters increased to significant ($P < 0.01$) levels during lactation, and an associated significant reduction occurred in viability and lactation indices. Pup weight was unremarkable directly after birth and up to day 4. After several days of lactation, it was observed that mean pup weight gain was significantly reduced ($P < 0.01$) at 0.40 mg/kg bw per day in both F_{1a} and F_{1b} litters on days 7, 14 and 21 (Table 13), a finding that was associated with increased numbers of pups that were thin, weak and cold to the touch. No treatment-related skeletal abnormalities occurred in pups dying during lactation.

The other fertility parameters evaluated, fertility index, duration of gestation, gestation index and sex ratio, were unaffected by treatment at all dose levels. Testis and epididymis weights of P generation animals were unaffected by treatment at all dose levels.

F_{1b} generation weanlings. Full postmortem examination, organ weight analysis and histopathological evaluation of five newly weaned F_{1b} pups of each sex per group revealed no treatment-related gross lesions other than the small size of male pups from parental animals treated at 0.40 mg/kg bw per day. There were no treatment-related organ weight changes in any group, although brain weight relative to body weight was elevated at 0.40 mg/kg bw per day as a result of growth retardation. There were no histopathological alterations in the reproductive organs at any dose level. Treatment-related retinal anomalies occurred in 3/4 males and 1/5 females at 0.40 mg/kg bw per day (Table 14). The anomaly was characterized by single or multiple retinal folds of many layers of the retina, which included the pigment epithelium in the centre. Some anomalies appeared as intra-retinal rosettes. The retinal lesion also occurred in one control female and in a male from each of the groups treated at 0.05 and 0.12 mg/kg bw per day.

The retinal anomaly is considered to represent a transient, reversible lesion, possibly related to growth retardation, as the high-dose F_{1b} adults that were of normal size had no retinal anomalies, and only a single incidence occurred in adult animals treated at 0.40 mg/kg bw per day.

F_{1b} generation post-weaning. There were no treatment-related deaths or clinical signs at any dose during the post-weaning administration period. However, two males, one male and one male treated at 0.05, 0.12 and 0.40 mg/kg bw per day, respectively, died or were killed in extremis after

Table 12. Treatment-related effects in the P generation

Parameter	Group mean values			
	0 mg/kg bw per day	0.05 mg/kg bw per day	0.12 mg/kg bw per day	0.40 mg/kg bw per day
Weight gain (g)				
Males, days 0–77	331	333	360*	377**
Males, days 0–259	482	477	508 (5.4%)	560* (16.2%)
Females, days 0–63	116	125	129* (11.2%)	146** (25.9%)
Effects on F_{1a} litters				
No. (%) of dams with liveborn pups	24 (96.0)	26 (100)	28 (100)	25 (100)
Incidence of total litter loss (%)	8.0[a]	3.8	0.0	28.0**
No. of liveborn pups / pup mortality (%)	300 / 6.3	300 / 2.7	330 / 3.0	275 / 42.2**
Lactation index (% survival, days 4–21)	99.5	100	99.2	52.7**
Pup weight (g) ± SD				
Day 1	6.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.5	6.3 ± 0.7
Day 4 (pre-cull)	8.7 ± 1.3	8.9 ± 1.3	9.0 ± 1.5	8.4 ± 1.4
Day 7	12.8 ± 1.2	13.8 ± 1.9	13.8 ± 1.8	10.4 ± 3.0**
Day 14	26.4 ± 2.3	27.5 ± 3.3	28.2± 3.1	17.8 ± 7.8**
Day 21	41.0 ± 3.6	42.6 ± 4.4	42.9 ± 4.6	27.6 ± 11.8**
Effects on P males during F_{1b} mating				
No. of males mating / no. of males cohabiting	25 / 28	26 / 29	27 / 29	19** / 30
Duration of cohabitation (days) ± SD	2.6 ± 2.7	4.1 ± 3.6*	4.4 ± 3.2**	7.2 ± 5.8**
Effects on P females during F_{1b} mating				
No. (%) of dams with prolonged inter-estrus[b]	1 (3.6)	0 (–)	0 (–)	4 (13.3*)
No. (%) of females mating	28 (100)	30 (100)	30 (100)	22 (73.3**)
Duration of cohabitation (days) ± SD	3.8 ± 4.8	4.3 ± 4.2	4.5 ± 3.4	9.5 ± 8.7*
Effects on F_{1b} litters				
No. (%) of dams with liveborn pups	21 (100)	22 (95.6)	26 (100)	16 (100)
Incidence of total litter loss (%)	0.0	0.0	0.0	25.0**
No. of liveborn pups / pup mortality (%)	250 / 2.0	253 / 1.98	303 / 3.96	169 / 33.1**
Lactation index (% survival, days 4–21)	98.0	98.5	99.2	60.0**
Pup weight (g) ± SD				
Day 1	6.2 ± 0.7	6.2 ± 0.6	6.2 ± 0.7	6.6 ± 0.8
Day 4 (pre-cull)	8.8 ± 1.5	8.8 ± 1.5	9.2 ± 1.3	9.0 ± 1.6
Day 7	13.8 ± 2.4	13.8 ± 2.4	14.5 ± 1.8	11.3 ± 3.1**
Day 14	28.9± 4.1	28.9 ± 4.1	29.6 ± 3.0	19.6 ± 9.4**
Day 21	45.1 ± 6.0	45.1 ± 3.4	46.1 ± 4.3	31.4 ± 14.4**

bw: body weight; F_1: first filial generation; P: parental generation; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$

[a] Includes one dam with stillborn litter.

[b] Finding that is not regarded as treatment related, but which explains the observed effects of number and duration of cohabitation during breeding for F_{1b}.

Source: Gordon (1984e)

Table 13. Treatment-related effects in the F_{1b} generation (post-weaning)

Parameter	Group mean values			
	0 mg/kg bw per day	**0.05 mg/kg bw per day**	**0.12 mg/kg bw per day**	**0.40 mg/kg bw per day**
Male body weight (g), day 0	76.4	72.6	73.8	52.6**
Male weight gain (g), days 0–28	187	183	187	159**
Male weight gain (g), days 0–272	600	604	595	651
Female body weight (g), day 0	70.0	68.3	64.5*	50.5**
Effects on F_{2a} litters				
No. (%) of dams with liveborn pups	27 (100)	23 (100)	25 (100)	23 (100)
No. of liveborn pups / pup mortality (%)	284 / 1.8	230 / 1.3	256 / 1.2	209 / 5.7**
Lactation index (% survival, days 4–21)	100	100	99.5	93.9**
Viability index (days 4[a]–14)	100	100	100	94.4**
Pup weight (g) ± SD				
Day 1	6.2 ± 0.7	6.2 ± 0.6	6.1 ± 0.6	6.3 ± 0.7
Day 4 (pre-cull)	8.4 ± 1.0	8.7 ± 1.0	8.8 ± 1.0	8.8 ± 1.0
Day 7	12.9 ± 1.4	13.3 ± 1.4	13.3 ± 1.2	12.0 ± 1.7*
Day 14	26.7 ± 2.5	27.1 ± 2.7	27.1 ± 2.3	20.6 ± 5.3**
Day 21	42.5 ± 3.9	42.9 ± 3.3	42.7 ± 3.4	32.9 ± 7.9**
Effects on F_{2b} litters				
No. (%) of dams with liveborn pups	21 (100)	12 (100)	21 (100)	17 (100)
No. of liveborn pups / pup mortality (%)	213 / 4.2	124 / 1.6	197 / 1.5	162 / 8.6**
Lactation index (% survival, days 4–21)	100	100	99.4	92.8**
Viability index (day 4[a]–14)	100	100	99.4	93.5**
Pup weight (g) ± SD				
Day 1	6.3 ± 0.7	6.5 ± 0.6	6.6 ± 1.1	6.3 ± 0.7
Day 4 (pre-cull)	8.7 ± 1.5	9.1 ± 1.4	9.5 ± 2.0	8.2 ± 1.1
Day 7	13.4 ± 2.3	13.7 ± 2.0	14.2 ± 2.5	11.7 ± 1.5*
Day 14	28.5 ± 4.2	27.3 ± 3.0	27.3 ± 4.4	20.7 ± 4.8**
Day 21	45.9 ± 6.5	42.5 ± 4.5	42.9 ± 7.2	33.2 ± 8.4**

bw: body weight; F_2: second filial generation; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$
[a] Post-cull.
Source: Gordon (1984e)

Table 14. Incidence of treatment-related retinal anomaly in F_{1b} weanlings and adults and F_{2b} weanlings

	Incidence of retinal anomaly							
	0 mg/kg bw per day		**0.05 mg/kg bw per day**		**0.12 mg/kg bw per day**		**0.40 mg/kg bw per day**	
Generation	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
F_{1b} weanlings	0/5	1/5	1/5	0/5	1/5	0/5	3/4	1/5
F_{1b} adults	0/8	0/25	0/10	0/25	0/10	0/25	0/10	1/25
F_{2b} weanlings	3/57	2/51	0/26	1/34	5/88	2/86	10*/63	18*/66

bw: body weight; F: female; F_1: first filial generation; F_2: second filial generation; M: male; *: $P < 0.05$
Source: Gordon (1984e)

29–262 doses. One female treated at 0.05 mg/kg bw per day died during gestation, after 189 doses. No gross or histopathological alterations occurred in the animals, and, as no dose–response relationship was evident, the deaths are considered incidental to treatment. Group mean body weights of males and females at 0.40 mg/kg bw per day and of females at 0.12 mg/kg bw per day were significantly reduced ($P < 0.05$ or $P < 0.01$) at the start of treatment, owing to retarded pre-weaning growth. Treatment-related reduced weight gain ($P < 0.01$) continued in males at 0.40 mg/kg bw per day for 4 weeks, after which weight gain was enhanced, such that terminal body weights were increased by 4.0%. There was no effect on weight gain in the other male groups or in any female groups during the premating, gestation and lactation periods for both the F_{2a} and F_{2b} generations.

At the time of weaning, effects in the F_{2a} and F_{2b} litters were generally less severe than those observed for the F_{1a} and F_{1b} litters. There were no treatment-related effects on the incidence of stillborn pups. However, in both F_{2a} and F_{2b} litters treated at 0.40 mg/kg bw per day, pup mortality was significantly increased ($P < 0.01$) during the course of lactation, and the associated viability and lactation indices were significantly decreased ($P < 0.01$). Pup weights in the high-dose group seemed to be unaffected by treatment directly after birth and for the first few days, but were significantly reduced ($P < 0.05$ or $P < 0.01$) from day 7 to day 21. This was associated with increased numbers of pups that were thin, weak and cold to the touch. These effects were not apparent at lower dose levels. All other fertility parameters for both F_{2a} and F_{2b} litters were unaffected by treatment at all dose levels. No treatment-related skeletal abnormalities occurred in pups dying during lactation. The other fertility parameters evaluated, fertility index, duration of gestation, gestation index, the incidence of stillborn pups and sex ratio, were unaffected by treatment at all dose levels. Testis and epididymis weights of F_{1b} generation animals were unaffected by treatment at all dose levels.

Full postmortem examination, organ weight analysis and histopathological evaluation of 10 males and 25 females per group of F_{1b} adults revealed no treatment-related gross or histomorphological alterations at any dose level other than a single case of retinal anomaly at 0.40 mg/kg bw per day.

F_{2b} generation weanlings. Full postmortem examination, organ weight analysis and histopathological evaluation of five newly weaned F_{2b} pups of each sex per group revealed no treatment-related gross lesions other than the comparably smaller size of pups from parental animals treated at 0.40 mg/kg bw per day. There were no treatment-related organ weight changes in any group, although brain weight relative to body weight was high at 0.40 mg/kg bw per day owing to growth retardation. There were no histopathological alterations in the reproductive organs at any dose level. Examination of the eyes of 98, 50, 164 and 119 animals in the groups treated at 0, 0.05, 0.12 and 0.40 mg/kg bw per day, respectively, revealed a treatment-related increased incidence of retinal anomalies in 10/63 males and 18/66 females at 0.40 mg/kg bw per day (Table 14). The characteristics of this anomaly were identical to those described previously. The retinal lesion occurred at much lower frequencies in the control group and the groups treated at 0.05 and 0.12 mg/kg bw per day, and the occurrence at these dose levels is considered not to be treatment induced. Although the literature describes this effect as a fixation artefact, the observed dose–response relationship and high incidence make this interpretation unlikely.

Directly after birth, all newborn pups showed no adverse treatment-related effects in terms of litter size, survival, viability, body weight and other reproductive parameters. This means that pups in the womb were fully protected against abamectin toxicity by the placental barrier. All the above-mentioned adverse effects observed at 0.40 mg/kg bw per day developed later after the start of lactation and are considered to be a direct consequence of the exposure of the pups to abamectin via the milk. Neonatal rats are particularly susceptible to abamectin toxicity caused by incomplete development of the P-glycoprotein element of the blood–brain barrier at this age. The retarded weight gain in both F_1 and F_2 generation progeny and the transient reduced weight gain in male F_{1b} generation parental animals are a late consequence of reduced weight gain during lactation as well as the increased pup mortality. The increased incidence of transient retinal anomaly in the eyes of F_1 and F_2 generation weanlings can also be explained as a secondary effect due to reduced body weight and reduced growth.

In view of the above, the NOAEL for the parental and reproductive toxicity of abamectin technical in rats was 0.40 mg/kg bw per day, the highest dose tested. For effects on the offspring, the NOAEL was 0.12 mg/kg bw per day, based on the occurrence of transient, reduced weight gain in male F_{1b} generation parental animals, increased pup mortality and retarded weight gain in both F_1 and F_2 generation progeny and an increased incidence of transient retinal anomaly (folds) in the eyes of F_2 generation weanlings at 0.40 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Gordon, 1984e).

(b) Developmental toxicity

Rats

In a dose range–finding study, five groups of 10 naturally mated female rats (CRCD strain, 16 weeks old, 218–288 g) were treated orally, by gavage, with abamectin technical (L-676,863-00V50, purity approximately 94%) in sesame oil at a dose of 0 (vehicle only), 0.25, 0.5, 1.0 or 2.0 mg/kg bw per day from day 6 to day 17 of gestation (day 0 of gestation being the day on which plug or sperm were observed in the vaginal smear). Abamectin technical has been shown to be stable in sesame oil for at least 24 hours at the concentrations used in the study. Samples of abamectin technical in sesame oil, prepared in the first and last weeks of treatment, were analysed for test concentration and found to be acceptable. The animals were observed for clinical signs at least once daily during the treatment period. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. The animals were killed on day 20, and their pregnancy status was established.

One female treated at 2.0 mg/kg bw per day was killed in a moribund condition on day 18 of gestation. Prior to sacrifice, the animal had lost weight, appeared weak and displayed tremors. All other animals survived to termination, and no other treatment-related clinical signs were apparent. There was a treatment-related increase in maternal weight gain during the treatment period of 15.2–25.8% at dose levels of 0.25, 0.5 and 1.0 mg/kg bw per day, but not at 2.0 mg/kg bw per day. The effect was statistically significant ($P < 0.05$) at 0.25 and 1.0 mg/kg bw per day, but is considered not to be an adverse effect of treatment. Pregnancy incidences were 90% in the control group and 90%, 80%, 100% and 90% in order of ascending dose level of abamectin technical.

The NOAEL for maternal toxicity was identified as 1.0 mg/kg bw per day, based on the occurrence of tremors and morbidity in one animal at 2.0 mg/kg bw per day in the dose range–finding study (Gordon, 1982a).

In the main study, four groups of 25 naturally mated female rats (CRCD strain, 10.5 weeks old, 206–269 g) were treated orally, by gavage, with abamectin technical (MK-0936, batch no. L-676,863-00V50, purity approximately 94%) in sesame oil at a dose level of 0 (vehicle only), 0.4, 0.8 or 1.6 mg/kg bw per day from day 6 to day 19 of gestation (day 0 of gestation being the day on which plug or sperm were observed in the vaginal smear). The animals were observed for clinical signs at least once daily during the treatment period.

Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. The animals were killed on day 20, and their pregnancy status was established. The ovaries were removed and fixed, and the number of corpora lutea was determined. The fetuses were removed from the uterine horns, individually weighed, sexed and examined for external abnormalities. The uterine horns were examined for the presence of resorptions (classified as early, middle, late or near fetal) and dead fetuses. Visceral examination by dissection was performed on every third fetus of each litter and on all externally abnormal fetuses. The head of every third fetus was fixed in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal abnormalities and variations. Litter data and maternal body weight data were analysed statistically by ANOVA or analysis of covariance (ANCOVA) using a least significant difference procedure after normalization of non-parametric data.

There were no deaths or treatment-related clinical signs at any dose level. Maternal weight gain from day 6 to day 14 of gestation was significantly increased ($P < 0.05$) by 15.8–21.1% at all doses of abamectin technical. The effect is considered to be treatment related, but is not an adverse effect. Thereafter, the weight gain of the treated groups was slightly lower than the control values. At sacrifice on day 20, the overall body weight gain was not significantly increased at any dose level.

There were no treatment-related effects on preimplantation loss, implantation number, incidences of resorptions and dead fetuses, mean fetal weight (sexes combined) or sex ratios at any dose level. The significantly ($P < 0.05$) higher incidence of resorptions in the group treated at 0.8 mg/kg bw per day is considered not to be treatment related, as more than 50% of the total number of resorptions occurred in two litters, and a similar effect was not apparent at 1.6 mg/kg bw per day.

Five externally malformed fetuses occurred: a control fetus with anophthalmia, two fetuses from different litters exposed to 0.8 mg/kg bw per day and a further two fetuses from different litters exposed to 1.6 mg/kg bw per day. At 0.8 mg/kg bw per day, one fetus had gastroschisis, and the other had multiple abnormalities, including anasarca, micrognathia, cleft palate and ectromelia. At 1.6 mg/kg bw per day, one fetus had cleft palate, and the other was a conjoined twin with exencephaly. None of the malformations is considered to be treatment related because of the isolated occurrence of each malformation and because cleft palate, gastroschisis and multiple malformation have occurred spontaneously in historical control groups. There was no evidence of developmental toxicity at any dose level, based on the incidence and nature of visceral and skeletal malformations and variations. Seven, four and three fetuses had one or more malformations in the groups treated at 0.4, 0.8 and 1.6 mg/kg bw per day, respectively, compared with two malformed control fetuses. The higher incidence in the lowest-dose group was due to the occurrence of three cases of hypoplastic rib and three cases of missing vertebrae in two litters. Either all visceral and skeletal malformations in the treated groups occurred in isolation or their distribution between the groups was not dose related. Slightly higher incidences of the variation distended ureter occurred in the treated groups, but higher incidences have occurred in historical control groups.

The incidences of the skeletal variations lumbar ribs and lumbar count variations were higher in the group treated at 1.6 mg/kg bw per day than in the control group. However, the litter distribution of these variations was comparable with the control group distribution (16 and two litters at 1.6 mg/kg bw per day compared with 13 and one control litters, respectively), and higher incidences have occurred in historical control groups. Therefore, none of the increased incidences of variations is considered to be treatment related.

In view of the above, the NOAEL for maternal toxicity and embryo/fetal toxicity for abamectin technical in rats was identified as 1.6 mg/kg bw per day, the highest dose tested. The minimal, transient and non-dose-related increase in maternal weight gain observed at all dose levels was not considered to be an adverse effect.

The study was GLP compliant, and a QA statement was attached (Gordon, 1982a).

Rabbits

In a dose range–finding study, four groups of 10 artificially inseminated female rabbits (New Zealand albino, 6–7 months old, weighing 3.64–4.60 kg) were treated orally, by gavage, with abamectin technical (MK-0936, batch no. L-676,863-00V50, purity approximately 94%) in sesame oil at a dose of 0 (vehicle only), 0.5, 1.0, 2.0 or 3.0 mg/kg bw per day from day 6 to day 18 of gestation (day 0 of gestation is the day of insemination). Abamectin mixtures at the concentrations used in the study have been shown to be stable in sesame oil for at least 24 hours. The animals were observed for clinical signs once daily throughout the study and twice daily during the treatment period. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18, 19 and 28 of gestation. The animals were killed on day 28, and their pregnancy status was established.

One female treated at 3.0 mg/kg bw per day was killed in a moribund condition on day 16 of gestation. Prior to sacrifice, the animal had lost weight and was prostrate, with laboured respiration and discharges from the nose and mouth. All other animals treated at 3.0 mg/kg bw per day survived

to termination but displayed stuporous behaviour 2–5 hours after the fourth and subsequent doses. Some animals in the group also showed a watery yellow-green discharge from the nose and mouth and reduced feed and water consumption (subjective assessment). No deaths or treatment-related clinical signs occurred at the lower dose levels. Four animals aborted prior to caesarean section: one treated at 1.0 mg/kg bw per day on day 20, one treated at 2.0 mg/kg bw per day on day 27, and two treated at 3.0 mg/kg bw per day on days 26 and 27 of gestation. This incidence of abortion is considered not to be treatment related, despite its absence in the control group, as spontaneous abortion has occurred in historical control groups. A statistically significant ($P < 0.05$) body weight loss of 9.5% occurred at 3.0 mg/kg bw per day during the treatment period, with some recovery after day 19; however, at sacrifice on day 28, an overall weight loss of 3.6% had occurred during the gestation period. There was no treatment-related effect on body weight at lower doses of abamectin. The pregnancy incidence was 100% in the control group and 70%, 80%, 90% and 90% in order of ascending dose level of abamectin technical (Gordon, 1982b).

In the main study, four groups of 18 artificially inseminated female rabbits (New Zealand albino, 6–7 months old, weighing 3.47–4.74 kg) were treated orally, by gavage, with abamectin technical (MK-0936, batch no. L-676,863-00V50, purity approximately 94%) in sesame oil at a dose of 0 (vehicle only), 0.5, 1.0 or 2.0 mg/kg bw per day from day 6 to day 27 of gestation (day 0 of gestation is the day of insemination). The animals were observed for clinical signs once daily throughout the study and twice daily during the treatment period. Body weights were recorded on days 0, 6, 9, 12, 15, 18, 21, 24, 27 and 28 of gestation. The animals were killed on day 28, and their pregnancy status was established. The ovaries were removed, and the numbers of corpora lutea were determined. The fetuses were removed from the uterine horns, individually weighed and examined for external abnormalities. All fetuses were killed and the viscera examined by dissection, at which time the sex was determined. The uterine horns were examined for the presence of resorptions (classified as early, middle, late or near fetal) and dead fetuses. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal malformations and variations. Litter data and maternal body weight data were analysed statistically by ANOVA or ANCOVA using a least significant difference procedure after normalization of non-parametric data.

Two deaths and one premature sacrifice occurred in abamectin-treated groups. One animal at 0.5 mg/kg bw per day died on day 26 of gestation, one animal at 1.0 mg/kg bw per day died on day 27 and one animal treated at 2.0 mg/kg bw per day was killed in a moribund condition on day 20. Death was preceded by reduced feed and water consumption in two animals and by blood-stained urine in the cage of the other animal. The relationship of these deaths to treatment with abamectin is equivocal, as a dose-related increase in incidence did not occur, six control animals also showed reduced feed and water consumption, and two controls had blood in the cage during the latter part of gestation. Premature abortion occurred in two females, one female and one female in the groups treated at 0.5, 1.0 and 2.0 mg/kg bw per day, respectively. This incidence of abortions is considered not to be treatment related, as one control animal also aborted. There were no clinical signs of toxicity at any dose level.

The feed consumption and water consumption of all groups were variable; by subjective assessment, the periods of reduced feed and water consumption in the group treated at 2.0 mg/kg bw per day were more prolonged and pronounced than in the other groups. This decreased feed and water consumption in females at 2.0 mg/kg bw per day resulted in a substantial weight loss during the dosing period that was statistically significant ($P < 0.05$) between days 6 and 18 of gestation, compared with controls. Females treated at 2.0 mg/kg bw per day had lost 3.3% of their weight at sacrifice on day 28 compared with the start of treatment. Body weight gain was unaffected by treatment at lower dose levels.

There were no treatment-related effects on preimplantation loss, implantation number, incidences of resorptions and dead fetuses, mean fetal weight (sexes combined) or sex ratios at any dose level. Significantly higher ($P < 0.05$) mean numbers of implantations and dead fetuses in the

group treated at 1.0 mg/kg bw per day are considered not to be treatment related, as there was no dose–response relationship.

One or more malformations occurred in four, four and 13 fetuses from females treated at 0.5, 1.0 and 2.0 mg/kg bw per day, respectively, compared with three abnormal control fetuses (Table 15). The external malformations in the high-dose group comprised two fetuses with cleft palate and two fetuses with omphalocele, all from a single litter, and five fetuses with clubbed forefeet from three other litters. The incidences of these malformations are higher than the concurrent and historical control group incidences and were considered treatment related. In addition, one fetus with clubbed forefeet had a lumbar vertebral malformation, and three of the fetuses in the litter with cleft palate and omphalocele had sternebral malformations, including one of the fetuses with cleft palate. The two other malformations occurring at 2.0 mg/kg bw per day, ventricular septal defect and branched rib, are considered not to be associated with treatment, as higher incidences have occurred in historical control animals. Two fetuses in a litter from a female treated at 1.0 mg/kg bw per day also had clubbed forefeet, but the occurrence is considered not to be treatment related, because higher incidences of the defect have been recorded in historical controls (one fetus from a concurrent control female also had a clubbed forefoot). The incidence and nature of malformations in fetuses from females treated at 0.5 and 1.0 mg/kg bw per day did not indicate an effect of treatment.

At 2.0 mg/kg bw per day, increased incidences of incomplete ossification of sternebrae and metacarpals are considered to reflect a slight treatment-related delay in ossification. A similar effect was not apparent at dose levels of 0.5 and 1.0 mg/kg bw per day.

A NOAEL of 1.0 mg/kg bw per day for maternal toxicity for abamectin technical in rabbits was identified, based on the occurrence of severe maternal toxicity (body weight loss) during gestation at 2.0 mg/kg bw per day. A NOAEL of 1.0 mg/kg bw per day for embryo and fetal toxicity was also identified, based on increased incidences of external malformations (cleft palate, omphalocele and clubbed forefeet) and incomplete ossification of sternebrae and metacarpals at 2.0 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Gordon, 1982b).

2.6 Special studies

(a) Acute neurotoxicity

Groups of 10 male and 10 female fasted Alpk:AP$_f$SD (Wistar-derived) rats (aged at least 42 days; males 305–362 g and females 197–244 g on the day prior to dosing) were administered abamectin (batch no. VS094KO, purity 96.2%) at a single oral dose of 0 (control), 0.5, 1.5 or 6 mg/kg bw and observed for the following 14 days. The control substance and vehicle for the test substance was sesame seed oil. All animals were observed prior to the start of the study and daily throughout the study for any changes in clinical condition. In addition, a functional observational battery, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, was performed in week −1 and on day 1 (at the time of peak effect), day 8 and day 15. Locomotor activity was also monitored in week −1 and on days 1, 8 and 15. Body weights and feed consumption were measured weekly throughout the study. At the end of the scheduled period, five rats of each sex per group were perfused in situ, brains were weighed and selected nervous system tissues were removed, processed and examined microscopically.

At 6 mg/kg bw, adverse clinical signs were evident at the time of peak effect, 6–7 hours post-dosing on day 1. These signs comprised reduced splay reflex in the majority of animals and splayed and tiptoe gait in one female. These signs were transient, although reduced splay reflex persisted on day 2 or 3 in some females. Motor activity was also reduced on day 1 in females at this dose, but this is consistent with the clinical signs present at this time. Brain weight was lower in females at this dose, but there was no microscopic change in the brain.

At 1.5 mg/kg bw, the only treatment-related effect was reduced splay reflex in two males and three females at the time of peak effect on day 1. There were no treatment-related effects on body

Table 15. Incidence of fetal malformations and variations in the main study on developmental toxicity in rabbits

Parameter	0 mg/kg bw per day	0.5 mg/kg bw per day	1.0 mg/kg bw per day	2.0 mg/kg bw per day	Historical control data for selected fetal alterations			
					No. of fetuses examined	No. of litters examined	No. of fetuses with alteration (%)	No. of litters with alteration (%)
External examination								
No. of fetuses (litters) examined	97 (15)	90 + 1[a] (13)	95 + 5[a] (11)	121 (15)	–	–	–	–
No. of fetuses (litters) with malformations	2 (2)	0 (0)	2 (1)	9 (4)	–	–	–	–
No. of fetuses (litters) with variations	0 (0)	0 (0)	0 (0)	0 (0)	–	–	–	–
No. of fetuses (litters) with cleft palate	0	0	0	2 (1)	–	–	–	–
No. of fetuses (litters) with omphalocele	1	0	0	2 (1)	–	–	–	–
No. of fetuses (litters) with clubbed forefoot	1	0	2 (1)	5 (3)	177	25	7 (4.0)	1 (4.0)
Visceral examination								
No. of fetuses (litters) examined	97 (15)	90 + 1[a] (13)	95 + 5[a] (11)	121 (15)	–	–	–	–
No. of fetuses (litters) with malformations	1 (1)	0 (0)	0 (0)	1 (1)	–	–	–	–
No. of fetuses (litters) with variations	3 (3)	2 (2)	3 (3)	3 (2)	–	–	–	–
No. of fetuses with ventricular septal defect[m]	0	0	0	1	989	135	3 (0.3)	3 (2.2)
No. of fetuses with retrocaval ureter[m]	1	0	0	0	–	–	–	–
No. of fetuses with variation in lung lobe[v]	1	2	2	3	–	–	–	–

Parameter	0 mg/kg bw per day	0.5 mg/kg bw per day	1.0 mg/kg bw per day	2.0 mg/kg bw per day	Historical control data for selected fetal alterations			
					No. of fetuses examined	No. of litters examined	No. of fetuses with alteration (%)	No. of litters with alteration (%)
No. of fetuses with azygous vein variation[v]	1	0	1	0	–	–	–	–
No. of fetuses with gallbladder reduced in size[v]	1	0	0	0	–	–	–	–
Skeletal examination								
No. of fetuses (litters) examined	97 (15)	90 + 1[a] (13)	95 + 5[a] (11)	121 (15)	–	–	–	–
No. of fetuses (litters) with malformations	0 (0)	4 (3)	2 (1)	5 (3)	–	–	–	–
No. of fetuses (litters) with variations	49 (12)	66 (9)	37 (10)	115 (12)	–	–	–	–
No. of fetuses with craniostenosis[m]	0	0	1	0	–	–	–	–
No. of fetuses with vertebral malformation[m]	0	3	1	1	84	10	2 (2.4)	2 (20)
No. of fetuses with branched rib[m]	0	1	0	1	177	25	2 (1.1)	2 (8.0)
No. of fetuses with fused ribs[m]	0	1	0	0				
No. of fetuses (litters) with sternebral malformation[m]	0	0	0	3 (1)	184	26	10 (5.4)	5 (19.2)
No. of fetuses with incompletely ossified vertebra[v]	1	0	0	0	–	–	–	–
No. of fetuses with sternebral variation[v]	1	0	0	0	–	–	–	–
No. of fetuses with incompletely ossified sternebra[v]	17	17	16	42	–	–	–	–

Table 15 (continued)

Parameter	0 mg/kg bw per day	0.5 mg/kg bw per day	1.0 mg/kg bw per day	2.0 mg/kg bw per day	Historical control data for selected fetal alterations			
					No. of fetuses examined	No. of litters examined	No. of fetuses with alteration (%)	No. of litters with alteration (%)
No. of fetuses with incompletely ossified pelvic bone[v]	1	2	0	3	–	–	–	–
No. of fetuses with incompletely ossified metacarpal[v]	8	15	7	33	–	–	–	–
No. of fetuses with incompletely ossified phalanx[v]	19	27	12	31	–	–	–	–
No. of fetuses with incompletely ossified talus calcaneous[v]	2	5	2	6	–	–	–	–
Total no. of malformed fetuses	3	4	4	13[b]	–	–	–	–
% malformed fetuses	3.09	4.40	4.00	10.7	–	–	–	–

bw: body weight; m: malformation; v: variation
[a] Dead fetus(es).
[b] Skeletal malformations partially in the same fetuses with external malformations.
Source: Gordon (1982b)

weight, feed consumption, grip strength, sensory perception, landing foot splay measurements or neuropathology.

In view of the above, it is concluded that single oral administration of abamectin at 6 mg/kg bw caused some clinical signs of neurotoxicity in males and females (principally reduced splay reflex) 6–7 hours after dosing on day 1, a reduction in motor activity in females on day 1 and slightly lower brain weights in females. Oral administration of abamectin at 1.5 mg/kg bw also caused a slightly reduced splay reflex in some males and females 6–7 hours after dosing on day 1. There was no neuropathology at abamectin doses up to 6 mg/kg bw.

The NOAEL in this study was 0.5 mg/kg bw, based on a reduced splay reflex at 1.5 mg/kg bw.

The study was GLP compliant, and a QA statement was attached (Brammer, 2006a).

(b) Subchronic neurotoxicity

Groups of 16 male and 16 female Alpk:APfSD (Wistar-derived) rats (approximately 42 days of age; weight range 252–325 g for males and 186–238 g for females at the start of dose

administration) were dosed orally (by gavage) with abamectin (batch no. VS094KO, purity 96.2%) at 0 (control), 0.4, 1.6 or 4.0 mg/kg bw per day for at least 90 consecutive days. The test substance was formulated in sesame seed oil without correction for purity, on a weekly basis.

All animals were observed prior to the start of the study and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed on at least 10 rats of each sex per group in weeks −1, 2, 5 and 9 and on all animals in week 14. Locomotor activity was also monitored on at least 10 rats of each sex per group in weeks −1, 2, 5 and 9 and on all animals in week 14. Body weights and feed consumption were measured and feed utilization was calculated weekly throughout the study. Urine samples, collected during week 13, were analysed. At the end of the study, five rats of each sex per group were killed by in situ perfusion fixation, brains were weighed and selected nervous system tissues were removed, processed and examined microscopically. All other surviving rats were killed and examined postmortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for histopathological examination.

Administration of abamectin at 4.0 mg/kg bw per day was generally tolerated well for up to 6 weeks, although there was evidence of toxicity characterized by lower body weight in females and adverse clinical signs in both sexes (irregular breathing, upward curvature of the spine, reduced righting reflex, reduced splay reflex and sides pinched in), which increased in incidence and severity with time. In addition, there were effects on hindlimb grip strength in females in weeks 2 and 5. During week 7, animals of both sexes lost weight over several days (from day 42) and showed a significant deterioration in clinical condition. The animals were therefore killed for humane reasons during week 7.

Oral administration of abamectin at 1.6 mg/kg bw per day caused no clinical signs of neurotoxicity, no significant clinical effects in the functional observational battery and no effects on landing foot splay, sensory perception (as shown in the tail flick test), forelimb grip strength or motor activity. There was no effect on perfused brain weight or treatment-related neuropathology.

There was no evidence of systemic toxicity in animals dosed with 0.4 or 1.6 mg/kg bw per day. There were no treatment-related clinical signs, no effects on body weight, feed consumption, ophthalmoscopic findings, haematology or clinical chemistry, or organ weights, and there was no treatment-related histopathology.

In view of the above, it is concluded that oral administration of abamectin at 4.0 mg/kg bw per day was generally well tolerated for up to 6 weeks, although there was evidence of toxicity characterized by a low incidence of adverse clinical signs in both sexes and lower body weight and lower grip strength in females. The animals showed a sudden and progressive weight loss and a significant deterioration in clinical condition during week 7, which necessitated removal of this group from the study.

The NOAEL for systemic toxicity and neurotoxicity was 1.6 mg/kg bw per day, based on reduced body weights in females, clinical signs in both sexes (irregular breathing, upward curvature of the spine, reduced righting reflex, reduced splay reflex) and changes in hindlimb grip strength in females at 4.0 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Brammer, 2006b).

(c) Developmental neurotoxicity

Groups of 30 female Alpk:AP$_f$SD (Wistar-derived) rats (time-mated, 10–12 weeks old, 220–300 g on delivery; actual body weight range 206–310 g) were given abamectin (batch no. VS094KO, purity 96.2%) by gavage at 0, 0.12, 0.20 or 0.40 mg/kg bw per day from day 7 of gestation through parturition and lactation to day 22 postpartum. The control substance and vehicle for the test substance was sesame seed oil. The day of sperm-positive vaginal smear was designated day 1 of gestation. Pups were allocated to the F_1 phase of the study on postpartum day 5, separated from the

dam on day 29 and allowed to grow to adulthood. Evaluations included observations to detect gross neurological and behavioural abnormalities, effects on motor activity, response to auditory startle, assessment of learning and memory, and neuropathological evaluation, including brain weights.

For the parental females, there were no treatment-related clinical observations or effects detected in the functional observational battery. There was a dose-related effect of treatment with abamectin on body weight during gestation. The body weights were significantly higher than those of the controls following the first or second dose. There was also a treatment-related effect on body weight postpartum, although the effect was not dose related for the 0.20 and 0.40 mg/kg bw per day groups. Following the last dose on day 22 postpartum, the difference in body weights between the control and abamectin-treated animals was reduced by day 29, indicating a recovery from the transient effect of abamectin.

For the abamectin-treated groups, maternal feed consumption was higher than in the control group during gestation through to day 5 postpartum.

There was no effect of treatment with abamectin on the proportion of pups born live, litter size on day 1 or the proportion of male pups. To day 5 postpartum, there was no effect of maternal treatment with abamectin on pup clinical condition, body weight or survival.

There was no effect of maternal treatment with abamectin on the clinical condition of the F_1 animals, and no treatment-related effects in the functional observational battery were detected at any time point.

For days 5–22 postpartum, the mean body weights of the F_1 males and F_1 females in the abamectin-treated groups were higher than those in the control group, although not in a dose-related manner. For days 29–63 postpartum, the body weights of the F_1 animals in the abamectin-treated groups were lower than those in the control group, although the differences from control values were statistically significant for the 0.20 and 0.40 mg/kg bw per day groups only (Table 16).

The mean day of age when vaginal opening occurred was statistically significantly later for the females in the 0.20 and 0.40 mg/kg bw per day groups, in comparison with the control group (Table 17).

Table 16. Intergroup comparison of F_1 body weights at selected time points

	Adjusted mean body weight (g)							
	Males				**Females**			
Day	**0 mg/kg bw per day**	**0.12 mg/kg bw per day**	**0.20 mg/kg bw per day**	**0.40 mg/kg bw per day**	**0 mg/kg bw per day**	**0.12 mg/kg bw per day**	**0.20 mg/kg bw per day**	**0.40 mg/kg bw per day**
5	9.4	10.2**	10.4**	10.1*	9.2	9.9*	9.7*	9.7
29	93.4	92.4	92.0	87.1**	88.3	86.5	84.1**	81.0**
63	350.1	342.7	340.0*	323.2**	219.3	212.7	208.6**	197.5**

bw: body weight; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)
Source: Moxon (2005)

Table 17. Intergroup comparison of selected developmental landmarks

Observation	**0 mg/kg bw per day**	**0.12 mg/kg bw per day**	**0.20 mg/kg bw per day**	**0.40 mg/kg bw per day**
Day of vaginal opening	35.5	36.5	37.4**	38.3**
Body weight at landmark (g)	125.4	127.6	128.6	126.8

bw: body weight; **: $P < 0.01$
Source: Moxon (2005)

There was no effect of maternal treatment with abamectin on locomotor activity, the auditory startle response or learning or memory of the F_1 animals at any time point.

There was no effect of maternal treatment with abamectin on brain weight or histology of the F_1 animals at 12 or 63 days of age.

In view of the above, it can be concluded that abamectin at dose levels of 0.12, 0.20 and 0.40 mg/kg bw per day was associated with higher maternal body weight and feed consumption during the dosing period. Treatment-related effects on the offspring were confined to the 0.20 and 0.40 mg/kg bw per day groups and included lower body weights post-weaning and hence a slight delay in the time of vaginal opening. There was no effect of abamectin on the function or morphology of the nervous system.

On the basis of the results of this study, there is no evidence of developmental neurotoxicity at abamectin dose levels of up to 0.40 mg/kg bw per day. The NOAEL for offspring toxicity was 0.12 mg/kg bw per day, based on reduction in body weight and delay in vaginal opening at 0.20 mg/kg bw per day and above.

The study was GLP compliant, and a QA statement was attached (Moxon, 2005).

In a supplementary developmental neurotoxicity study performed to provide brain morphometry data, groups of 30 time-mated female Alpk:AP$_f$SD (Wistar-derived) rats were given abamectin by gavage at 0, 0.12, 0.20 or 0.40 mg/kg bw per day from day 7 of gestation through parturition and lactation to day 22 postpartum. The control substance and vehicle for the test substance was sesame seed oil. The day of sperm-positive vaginal smear was designated day 1 of gestation. Pups were allocated to the F_1 phase of the study on postpartum day 5, separated from the dam on day 29 and allowed to grow to adulthood. Evaluations included observations to detect gross neurological and behavioural abnormalities, effects on motor activity, response to auditory startle, assessment of learning and memory, and neuropathological evaluation, including morphometry and brain weights.

Maternal treatment with 0.40 mg/kg bw per day was associated with high pup mortality, resulting in an insufficient number of litters/pups available to meet the objectives of the study. All dams and pups in this group were therefore removed from the study (between days 15 and 38 postpartum) prior to scheduled termination.

For the parental females, there were no treatment-related deaths, clinical observations or effects detected in the functional observational battery.

There was a treatment-related increase in body weight and feed consumption during gestation; the effect was dose related. There was also a treatment-related effect on body weight on day 1 postpartum, with higher mean values for the 0.20 and 0.40 mg/kg bw per day groups, although there was no effect on subsequent days. For days 5–23 postpartum, feed consumption was lower for the 0.40 mg/kg bw per day group. There was no effect of 0.12 or 0.20 mg/kg bw per day on feed consumption postpartum.

There was no effect of treatment with abamectin on the proportion of pups born live, litter size on day 1 or the proportion of male pups. To day 5 postpartum, there was no effect of maternal treatment with abamectin on clinical condition or survival of pups, although the body weight of the males in the 0.40 mg/kg bw per day group was lower than that of the controls. A similar difference was not seen in females.

After day 5, maternal treatment with 0.40 mg/kg bw per day was clearly associated with toxicity, and the pups showed signs of dehydration, tremors and lower body weights, resulting in the premature death of 31% (59/190) of the animals. The group was therefore terminated prematurely.

There was no effect of maternal treatment with abamectin at 0.12 or 0.20 mg/kg bw per day on the clinical condition of the F_1 animals, and no treatment-related effects were detected in the functional observational battery at any time point.

Table 18. Intergroup comparison of F_1 body weights at selected time points

	Adjusted mean body weight (g)							
	Males				Females			
Day	0 mg/kg bw per day	0.12 mg/kg bw per day	0.20 mg/kg bw per day	0.40 mg/kg bw per day	0 mg/kg bw per day	0.12 mg/kg bw per day	0.20 mg/kg bw per day	0.40 mg/kg bw per day
5	9.3	9.5	10.1**	9.6	9.0	9.2	9.6*	9.4
8	15.4	15.3	15.2	13.1**	14.8	15.0	14.8	12.8**
29	91.5	87.3*	86.5*	61.6**	87.5	81.9**	81.1**	55.6**
63	354.6	335.2**	326.6**	354.6	218.7	206.1**	202.9**	218.7

bw: body weight; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)
Source: Moxon (2007)

Table 19. Intergroup comparison of vaginal opening

Observation	0 mg/kg bw per day	0.12 mg/kg bw per day	0.20 mg/kg bw per day
Day of vaginal opening	32.0	33.3*	33.3*
Body weight at landmark (g)	102.0	102.7	101.7

bw: body weight; *: $P < 0.05$
Source: Moxon (2007)

For the pups in the 0.12 and 0.20 mg/kg bw per day groups, mean body weights were slightly lower than those of the control group from day 29 through to day 63, and the decrease was dose related (Table 18). For this reason, a slight delay in the time of vaginal opening was also observed (Table 19). There was no effect of treatment on the age when preputial separation occurred.

There was no effect of maternal treatment with abamectin at 0.12 or 0.20 mg/kg bw per day on locomotor activity, auditory startle response, learning or memory of the F_1 animals at any time point.

There was no effect of maternal treatment with abamectin at 0.12 or 0.20 mg/kg bw per day on brain weight, histopathology or morphometry in the F_1 animals on day 12 or 63.

In conclusion, abamectin was evaluated at dose levels of 0.12, 0.20 and 0.40 mg/kg bw per day in this study, although the highest dose level of 0.40 mg/kg bw per day was toxic to the offspring following maternal exposure and resulted in the premature termination of the selected offspring in this group. All dose levels were associated with higher maternal body weight and feed consumption during gestation. For the 0.12 and 0.20 mg/kg bw per day groups, slightly lower body weights were observed post-weaning, with an associated slight delay in the time of vaginal opening. There was no effect of abamectin on the function or morphology of the nervous system. Although there was a reduction in body weights of offspring at all doses, the days of vaginal opening were within the normal range.

Under the conditions of this study, at 0.12 and 0.20 mg/kg bw per day, body weights of pups post-weaning were statistically significantly lower and the time of vaginal opening was statistically significantly later compared with the control group. However, the effects observed at 0.12 mg/kg bw per day were marginal and within the range of normal biological variation and were therefore not considered to be toxicologically significant. Hence, the NOAEL for offspring toxicity was 0.12 mg/kg bw per day. There was no effect on function or morphology of the nervous system at dose levels up to 0.40 mg/kg bw per day, the highest dose tested.

The study was GLP compliant, and a QA statement was attached (Moxon, 2007).

(d) Acute oral and reproductive toxicity in CF-1 mice

Acute oral toxicity studies in CF-1 mice

Acute oral toxicity and exploratory acute oral toxicity studies with abamectin in CF-1 mice are summarized in Table 20.

Table 20. Summary of acute oral toxicity and exploratory acute oral toxicity studies with abamectin

Species	Strain	Sex	Study	Dose (mg/kg bw)	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse[a]	CF-1	Female pregnant	Acute oral	0, 5, 10, 20, 40, 80	94	11.8	Gordon (1986b)
Mouse[b]	CF-1	Female non-pregnant	Acute oral	0, 5, 10, 20, 40, 80	94	15.0	Gordon (1986b)
Mouse[c]	CF-1	Female genotyped as homozygous positive (+/+)	Exploratory acute oral for expression of P-glycoprotein	0, 10, 20, 40, 80	Not specified	28	Hall (1997)
Mouse[c]	CF-1	Female genotyped as heterozygous (+/–)	Exploratory acute oral for expression of P-glycoprotein	0, 10, 20, 40, 80	Not specified	14	Hall (1997)

bw: body weight; LD_{50}: median lethal dose

[a] Symptoms included tremors, bradypnoea and clonic convulsions.

[b] Symptoms included loss of righting reflex and bradypnoea.

[c] Symptoms included tremors, bradypnoea and decreased activity. Death occurred at 40 mg/kg bw.

Ten-day reproductive toxicity study in CF-1 mice

Four groups of 20 naturally mated, singly housed, female mice (albino CF-1 strain, 12 weeks old, weight range 26.8–33.4 g) were treated orally with a mixture of tritiated (< 0.5% total) and unlabelled abamectin technical (MK-0936, batch nos L-676,895-01S25 and L-676,895-00V54, purity > 98% and > 88%, respectively), by admixture in the diet, at a constant concentration of 0 (vehicle only), 0.33, 1.0 or 2.0 ppm from day 6 to day 15 of gestation (day 0 being day on which vaginal plug observed). Target dose levels were 0, 0.1, 0.3 and 0.6 mg/kg bw per day, which were achieved (0, 0.10, 0.33 and 0.61 mg/kg bw per day, respectively) on days 6–11. However, on days 12–16 of gestation, as a result of lower feed consumption, achieved dose levels were reduced to 0, 0.06, 0.16 and 0.33 mg/kg bw per day, respectively. The individual dose levels achieved from day 12 to 16 in the group treated at 0.1 mg/kg bw per day ranged from 0.04 to 0.13 mg/kg bw per day, with three animals receiving 0.1 mg/kg bw per day during this period.

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 feed consumption was measured. The animals were killed on day 17 of gestation, and the unopened uteri were examined to estimate the numbers of implantation sites, resorptions and live fetuses. Dead fetuses were classified as resorptions. Maternal feed consumption and body weight data were analysed statistically by ANOVA or ANCOVA using a least significant difference procedure after normalization of non-parametric data by the Rankit method.

Two females treated at 0.6 mg/kg bw per day were killed in poor condition after 3 days of treatment, having developed marked tremors. Three females treated at 0.3 mg/kg bw per 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 0.1 mg/kg bw per day. There were no treatment-related effects on maternal weight gain during gestation at any dose level. However, the mean weight gains of all treated groups were significantly higher ($P < 0.05$) than the control values from day 16 to day 17, resulting in a treatment-related increase in terminal body weights of 3.1–5.5%.

There were no consistent treatment-related effects on feed consumption and no treatment-related effects on reproductive status, as assessed by pregnancy incidence, number of implantations and numbers of live fetuses and resorptions.

In view of the above, a NOAEL for maternal toxicity of 0.1 mg/kg bw per day (time-weighted average 0.08 mg/kg bw per day) for abamectin technical was identified, 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 per day. A NOAEL for reproductive parameters (pregnancy incidence, number of implantations and fetal viability) of 0.6 mg/kg bw per day (time-weighted average 0.48 mg/kg bw per day), the highest dose tested, was identified.

The study was GLP compliant, and a QA statement was attached (Gordon, 1984f, 1986a).

(e) Comparison of abamectin sensitivity and P-glycoprotein levels in CF-1 and CD-1 mice

To identify "abamectin-sensitive" and "abamectin-insensitive" CF-1 mice, two groups of mice (49 male and 50 female CF-1 strain mice and five male and five female CD-1 strain mice) were treated orally, by gavage, with abamectin (batch no. L-676,863-000V093, purity 96.0%) at 0.8 mg/kg bw per day as a solution in sesame oil at a treatment volume of 5 mL/kg bw for 4 days. Two further groups (five male and five female CF-1 strain mice and five male and five female CD-1 strain mice) received sesame oil at 5 mL/kg bw per day for 4 days, as vehicle control groups. All animals were weighed pre-dosing and observed for signs of neurotoxicity several times each day. Animals that showed severe tremors and/or ataxia at this dose level were killed and exsanguinated. A total of three vehicle control mice (one male and two 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 in isopentane/liquid nitrogen for immunohistochemical analysis of P-glycoprotein by an adaptation of a published method (Schinkel et al., 1994). Following staining with diaminobenzidine and haematoxylin, the slides were microscopically examined blind for relative staining intensity of P-glycoprotein against control sections of each tissue. Further samples of brain (cerebrum and cerebellum) and small intestine were cooled by ice for subsequent sodium dodecyl sulfate–polyacrylamide gel electrophoresis (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 Gerlach et al. (1987). Following completion of the immunohistochemical studies, five insensitive CF-1 strain mice of each sex per group and a random sample of naive CD-1 strain mice (five of each sex or 10 females per group) were treated with single oral doses of abamectin ranging from 1.0 to 10 mg/kg bw to determine their sensitivity to abamectin for comparison with the sensitive CF-1 subgroup.

Treatment with abamectin at 0.8 mg/kg bw per day induced clinical signs of neurotoxicity, whole-body tremors and slight ataxia only in CF-1 strain mice. The signs first occurred within 1.5 hours of treatment on day 1. Within 4 hours of treatment, 12 female and five male CF-1 mice, 17% of those tested, showed severe signs of toxicity, comprising dyspnoea, lateral recumbency, tremors and coma following handling. These animals, identified as sensitive to abamectin toxicity, were killed and subjected to necropsy, together with two females and one male from each of the other groups. No clinical signs of toxicity occurred in any of the other CF-1 strain mice during the 4-day treatment period.

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 in this strain.

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

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

In view of the above, it is concluded that 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 than P-glycoprotein-deficient individuals to abamectin toxicity.

The study was not GLP compliant (Lankas, 1994).

(f) P-glycoprotein developmental expression in rat pups

Exploratory study of P-glycoprotein developmental expression in rat pups

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 F_0 females and four unmated females assigned to this study (approximately 10 weeks of age; weight range 200–300 g). None was 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 F_0 females was checked daily. Body weights and feed consumption were not recorded. Scheduled live fetuses from F_0 females were weighed, sexed and given external examinations. Selected F_1 offspring were sexed and examined externally on postnatal day 0 and weighed at sacrifice.

Four F_0 females were sacrificed by carbon dioxide asphyxiation on gestation day 20. Within each litter, one male and one female fetus were terminated by rapid induction of hypothermia, and the brain and jejunum were snap frozen in isopentane/liquid nitrogen and cryosectioned for immunohistochemical analysis for P-glycoprotein. In addition, frozen samples of brain and jejunum were stored and later analysed for P-glycoprotein by western blot analysis. The brain, jejunum and uterus of the four dams were sampled, sectioned and analysed as above, immunohistochemically and by western blot technique.

F_1 offspring. On postnatal day 0, litters were culled to 10 pups each (five of each sex, fostering as needed). 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 of each sex per day) were sacrificed by rapid induction of hypothermia (days 2 and 5) or carbon dioxide asphyxiation (days 8, 11, 14, 17 and 20) and sampled and sectioned as described above for immunohistochemical analysis of P-glycoprotein in the brain and jejunum. Selected samples were analysed for P-glycoprotein by western blot analysis. To ensure random, unbiased sample collection, pups were selected such that they were sourced from as many litters as possible.

Four adult non-pregnant females were terminated by carbon dioxide asphyxiation, and the uteri were sampled, sectioned and analysed as described above for pregnant females.

The frozen sections were processed immunohistochemically using methods adapted from those of Schinkel et al. (1994) for P-glycoprotein. Slides were examined microscopically using a blind procedure and scored for relative staining intensity for P-glycoprotein.

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. A molecular dynamics scanning densitometer using a 5-minute film exposure with chemiluminescent detection was used.

There were no deaths or abortions during the study. External examination of fetuses 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 cells. 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. F_1 pups of all ages, including the gestation day 20 fetuses, showed P-glycoprotein staining in the brain. Expression appeared weaker in the gestation day 20 fetuses and younger pups compared with the older postnatal days 17 and 20 pups. However, P-glycoprotein staining of the jejunal epithelial cells was not apparent until approximately postnatal day 8. Minimal to very slight staining was seen in the postnatal day 8 pups' intestinal epithelial cells. P-glycoprotein staining appeared more intense in the older pups by approximately postnatal day 17, but did not achieve the intensity found in the F_0 adult females, even by postnatal day 20. The intensity of the P-glycoprotein seen microscopically in the F_1 pups was further supported by western blot analysis.

Many of the pups approximately 8 days old or younger did not have P-glycoprotein staining along the jejunal epithelial brush border. The absence of intestinal P-glycoprotein in these young pups may allow greater absorption of drugs, which suggests that higher plasma drug levels may be possible. Moreover, it can be hypothesized that higher plasma drug levels, coupled with less P-glycoprotein in the blood–brain barrier, may produce higher drug levels in the brain, resulting in clinical evidence of neurotoxicity, including death. Less abamectin may be absorbed from the intestine, and there is less accumulation in the brain, as the P-glycoprotein barrier develops in the intestinal tract and brain. Thus, it appears that young rat pups (8 days old) are potentially more susceptible to avermectin-related neurotoxicity because of differences in the development of the P-glycoprotein transport system. These results may explain the enhanced neonatal toxicity of avermectins to rats and the postnatal development of a blood–brain barrier to these substances in this species.

Quantification by scanning densitometry of western blots of brain samples shows that levels of P-glycoprotein in the gestation day 20 fetus are very low (approximately 11% of the adult value), as are levels in neonatal rats through to postnatal day 11. The apparent increase in the level for the rat fetus (11% of the adult value) compared with the early postnatal samples (5% of the adult value) is probably a reflection of qualitative differences of the protein as a function of age. The western blots show that the rat fetal protein migrates in a more diffuse pattern compared with the neonatal samples. As this protein undergoes significant post-translational processing (i.e. glycosylation), it is possible that the fetal 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 with 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.

In view of the above, it was concluded that the blood–brain barrier, formed by 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 not fully developed in neonatal rats. This barrier 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. Neonatal rats, with limited or no blood–brain barrier, have increased susceptibility to avermectin toxicity (Cukierski, 1995).

Examination of developmental expression of P-glycoprotein levels in rat pups

To examine the expression and localization of P-glycoprotein in the brain of adult rats and neonatal rat pups during their early development, female rats (Crj:Wistar) pregnant for 19 days were anaesthetized under ether inhalation, and rat fetuses were removed from the uteri by caesarean sections. Rats on postnatal days 1, 3, 7, 14, 21, 28, 56 and 84 were anaesthetized under ether inhalation, and the brains were dissected. Five rats were used at each time period. Brains were homogenized and separated by centrifugation into a cytosolic fraction (supernatant) and a membrane fraction (pellet). Aliquots were separated by SDS-PAGE, and P-glycoprotein was marked by a specific antibody (anti-human P-glycoprotein antibody C-19, Santa Cruz, CA, USA). For quantitative analysis, the X-ray film was scanned with a charge-coupled device colour scanner and then analysed by image analysis. The protein levels of P-glycoprotein determined were presented as the percentage of the protein level in the adult rat brain (postnatal day 84 = 12 weeks).

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

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

Although P-glycoprotein was detected in the adult rat brain, it was not detectable in the early stages of postnatal development in the cerebral cortex, cerebellum or hippocampus. P-glycoprotein was first detected from postnatal day 7 in the membrane fraction of all three areas of the brain. From postnatal day 7 onwards, levels gradually increased, reaching a plateau on postnatal day 28. The observed expression profile of P-glycoprotein in the membrane fraction was similar in the cerebral cortex, hippocampus and cerebellum.

Immunolocalization 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 (a marker for astrocytes) showed that P-glycoprotein was co-localized with the capillaries, but not the astrocytes.

These findings suggest that P-glycoprotein may act as part of the blood–brain barrier, and its expression may increase synchronously with other blood–brain barrier components: namely, formation of the tight junction–based endothelium and inclusion of other energy-dependent transport systems.

It can be concluded that adult rats show intense P-glycoprotein immunoreactivity. The expression and localization of P-glycoprotein in the brain during the early development of rats were examined. 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 by day 28 at levels approximating those seen in adult rats. There is evidence that P-glycoprotein expression increases with postnatal development and is localized in the brain capillaries, suggesting a role for P-glycoprotein in the blood–brain barrier.

The study was not GLP compliant (Matsuoka et al., 1999).

(g) Oral toxicity and plasma level study in monkeys

In an in-house investigative study, two groups of two male and two female rhesus monkeys (*Macaca mulatta*, 2–3 years of age, weight range 2.4–3.2 kg, supplied by Charles River Primates, USA) were treated orally, by gavage, with repeated single doses of abamectin (MK-0936, batch no. L-676,863-00V50, purity 94% by HPLC) or ivermectin (MK-0933, batch no. L-640,471-000W076, purity 97.3%) in sesame oil at 2- to 3-week intervals for a total of 13 doses. The dosing schedule was as follows: weeks 1, 4 and 6: 0.2 mg/kg bw; week 8: 0.5 mg/kg bw; week 10: 1.0 mg/kg bw; week 13: 2.0 mg/kg bw; week 15: 4.0 mg/kg bw; week 17: 2.0 mg/kg bw; week 19: 6.0 mg/kg bw; week 21: 8.0 mg/kg bw; week 24: 8.0 mg/kg bw; week 27: 12.0 mg/kg bw; and week 29: 24.0 mg/kg bw.

After the first two doses of either abamectin or ivermectin, treatment of the groups was crossed over in order to elucidate slight pupil dilation in two animals treated with ivermectin. Thereafter, the groups continued to receive the same test substance. Formulations prepared for each week, with the exception of week 4, were analysed for test substance concentration. All formulations, with the exception of the week 24 ivermectin sample, which had a mean concentration of 84% of the nominal concentration, were within 10% of the nominal concentration. Clinical signs were recorded daily. Feed consumption was estimated 5 times/week. Body weights were recorded twice each week up to week 9 and weekly thereafter. Ocular examinations for mydriasis were performed using a penlight light source pre-dosing and at 2, 4 and 24 hours post-dosing for all doses except 24.0 mg/kg bw (for which 8- and 48-hour examinations were also included). Plasma samples for analysis of test substance concentration were prepared from blood withdrawn 15 and 30 minutes and 1, 2, 4, 8, 24, 48, 72 and 96 hours following treatment at 2.0 mg/kg bw in week 17. Plasma samples were also taken 8, 24 and 48 hours after treatment at 8.0 mg/kg bw in week 24 and 8, 24, 48, 72 and 96 hours following treatment at 24.0 mg/kg bw in week 29. Analysis of plasma for abamectin or ivermectin concentration was performed by the modified liquid chromatography–fluorescence method.

Both abamectin and ivermectin produced emesis at 2.0 mg/kg bw, with a dose-related increase in incidence and decrease in time of onset. No treatment-related clinical signs occurred at doses less than 2.0 mg/kg bw. Therefore, emesis is considered to be the most sensitive indicator of toxicity in rhesus monkeys and was used to determine the minimum toxic dose. The minimum toxic dose (LOAEL) for both test substances is considered to be 2.0 mg/kg bw. Mydriasis is considered to be a less sensitive indicator of toxicity because a marked effect occurred only in response to abamectin at 24.0 mg/kg bw and because of interanimal variability. Pupil dilation and/or decreased pupil constriction occurred at dose levels of 6.0 mg/kg bw for abamectin and 12.0 mg/kg bw for ivermectin, but there was no relationship between dose level and time of onset and duration of effect. The incidence of mydriasis was higher in the group treated with abamectin than in the ivermectin-treated group. Three animals in each group displayed transient reduced activity or sedation after treatment at 24.0 mg/kg bw. The effect had a variable time of onset and persisted for up to 25 hours. The occurrence and severity of sedation or reduced activity did not correlate with mydriatic effects. There were no other treatment-related clinical signs and no effect on feed consumption or body weight at any dose level in either group.

Plasma concentrations of abamectin and ivermectin were similar for 4 hours after a dose of 2.0 mg/kg bw, but thereafter the plasma concentrations of ivermectin were generally higher than those of abamectin.

The peak plasma concentrations of abamectin and ivermectin occurred 4–24 hours and 8–48 hours post-treatment, respectively. The shapes of the plasma concentration–time plots suggested that the peak concentrations occurred between 8 and 24 hours for both compounds. The highest plasma concentrations were recorded 8 hours after treatment with doses of 8.0 and 24.0 mg/kg bw for both compounds. At 8, 24 and 48 hours after treatment with abamectin, the ratios of plasma concentrations between 2.0 and 8.0 mg/kg bw were 2.0, 0.8 and 2.5, respectively. The equivalent values for ivermectin were 2.8, 1.4 and 1.5, respectively. The ratios of plasma concentrations between 8.0 and 24.0 mg/kg bw at 8, 24 and 48 hours after treatment were 2.6, 4.2 and 3.4, respectively, for abamectin and 2.5, 2.6 and 3.6, respectively, for ivermectin. Therefore, there was an approximate proportionality between dose and plasma concentration for both compounds. There was a poor correlation between plasma levels in individuals and the occurrence of emesis and the incidence and severity of mydriasis.

Clinical signs of toxicity elicited by abamectin and ivermectin are confined to emesis and mydriasis at dose levels up to 12.0 mg/kg bw and, additionally, sedation at 24.0 mg/kg bw. The most sensitive indicator of abamectin and ivermectin toxicity in rhesus monkeys is emesis, as clinical signs of toxicity seen in mice and rats (tremors and convulsions) did not occur. The minimum toxic dose (LOAEL) of both abamectin and ivermectin was 2.0 mg/kg bw, and the NOAEL was 1.0 mg/kg bw. The maximum plasma concentrations of both abamectin and ivermectin occurred between 8 and 24 hours after oral administration. Plasma concentrations increased with increasing dose, but generally less than proportionally with dose. Approximate 5-fold and 7-fold increases in the plasma

concentrations of abamectin and ivermectin, respectively, over those produced by the minimum toxic dose did not elicit more severe clinical signs of toxicity, other than slight to moderate sedation.

The study was GLP compliant, and a QA statement was attached (Gordon, 1985c).

(h) Studies on metabolites

The 2000 JMPR defined the residue for compliance with maximum residue limits (MRLs) for animal commodities as the sum of avermectin B_{1a} and 8,9-*Z*-avermectin B_{1a}. The residue for compliance with MRLs for plant commodities and for estimating dietary exposure was defined as the sum of avermectin B_{1a}, avermectin B_{1b}, 8,9-*Z*-avermectin B_{1a} and 8,9-*Z*-avermectin B_{1b}. 8,9-*Z*-avermectin B_{1a} (also referred to as 8,9-*Z*-B_{1a}, Δ-8,9-isomer, NOA 427011 and L-652,280) and 8,9-*Z*-avermectin B_{1b} are the photodegraded products of abamectin. 8,9-*Z*-avermectin B_{1b} is present in smaller proportions and is assumed to have toxicological properties similar to those of 8,9-*Z*-B_{1a}.

Acute toxicity of 8,9-Z-B_{1a}

Studies on the acute toxicity of 8,9-*Z*-B_{1a} are summarized in Table 21.

Genotoxicity of 8,9-Z-B_{1a}

8,9-*Z*-B_{1a} (batch no. L-652,280-000N005, purity 91.6%) in dimethyl sulfoxide solvent was tested in four histidine-auxotrophic strains (TA97a, TA98, TA100 and TA1535) of *Salmonella typhimurium* and three tryptophan-auxotrophic strains of *Escherichia coli* (WP2, WP2 uvrA and WP2 uvrA pKM101) by preincubation for 48 hours at concentrations of 0 (solvent control), 10, 30, 100, 300, 1000 and 3000 µg/plate.

8,9-*Z*-B_{1a} did not produce any 2-fold or greater dose-related increase in the number of revertant colonies at any dose level in any of the strains tested. There was no inhibition of the background lawn growth of any strain at any dose level. Precipitation occurred on all plates at 3000 µg/plate. The positive control materials produced the expected increase in the numbers of revertant colonies in all strains.

In view of the above, 8,9-*Z*-B_{1a} and/or its metabolites did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study at doses up to 3000 µg/plate, a concentration at the limit of solubility.

The study was GLP compliant, and a QA statement was attached (Gordon, 1988c).

One-generation reproductive toxicity of 8,9-Z-B_{1a}

Four groups of 20 female Sprague-Dawley rats (Crl:CD(SD) BR strain, approximately 10 weeks old, weight range 192–242 g) were treated orally, by gavage, for 15 days prior to mating, throughout cohabitation and gestation, and through lactation until day 20 with 8,9-*Z*-B_{1a} (batch no. L-652,280-000N005, purity 91.6%) as a solution in sesame oil at a dose level of 0 (vehicle only), 0.06, 0.12 or 0.40 mg/kg bw per day. Individual doses were based on the most recent body weight. All maternal animals were observed for clinical signs twice daily, one observation being 1–5 hours after treatment. Maternal body weights were recorded weekly during the premating 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 feed consumption was measured at 5-day intervals twice during premating, 3 times during gestation and twice during lactation. After 15 days of treatment, the females were paired 1:1 with untreated males of the same strain for a maximum of 14 days. Vaginal lavage was performed daily to detect sperm (day 0 of gestation = the day on which sperm were observed in the vagina). The females were allowed to litter naturally, with observation of parturition behaviour 4 times daily from day 21 to day 24. 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 eight pups per litter on day 3 of lactation by random selection, but with a balanced sex ratio

Table 21. Summary of acute toxicity studies with 8,9-Z-B_{1a}, a metabolite of abamectin

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	CF-1	M + F	Oral[a]	> 99	> 5 000	Gordon (1984g)
Mouse	CF-1	M + F	Oral[b]	> 99	> 48	Gordon (1984g)
Mouse	Crl:CF1 (BR)	M + F	Oral[c]	> 99	> 80	Gordon (1986c)
Mouse	CD-1	F	Oral[d]	Not specified	217	Lynch (1996)
Mouse	CF-1	M	Oral[e]	Not specified	20	Lynch (1996)

bw: body weight; F: female; LD_{50}: median lethal dose; M: male

[a] Tested at dose levels of 1250, 2500 and 5000 mg/kg bw (polar photodegradate of abamectin). No deaths occurred in the animals treated at any dose level, and clinical signs were confined to the group treated at 5000 mg/kg bw. All mice in the group showed decreased activity and bradypnoea within 10 minutes of treatment, and this persisted for approximately 4 hours. All animals, except one female, gained weight during the subsequent observation period. There were no treatment-related gross changes at necropsy.

[b] Tested at dose levels of 6, 12, 24 and 48 mg/kg bw (non-polar metabolite). One male treated at 12 mg/kg bw died approximately 4.5 hours after treatment. Prior to death, the animal had decreased activity and bradypnoea; at necropsy, the cause of death was established as dose maladministration. Treatment-related clinical signs were absent in all dose groups, but the sesame oil controls and the groups treated at 24 and 48 mg/kg bw showed slight perianal oil staining. One female treated at 6 mg/kg bw had pale extremities and had lost weight on day 14. At necropsy, the animal had a blood clot on the ovary. All other animals, except one female, gained weight during the observation period. No treatment-related gross changes were evident at necropsy in any dose group.

[c] Tested at dose levels of 0, 5, 10, 20, 40 and 80 mg/kg bw. Clinical signs of toxicity, including decreased activity, bradypnoea, ataxia and ptosis, occurred within 60–90 minutes of treatment in all treated groups except for females receiving 5 mg/kg bw. The symptoms persisted until day 2 in both sexes treated at 80 mg/kg bw and in the males treated at 40 mg/kg bw. Mortality incidences were 10%, 10%, 20%, 0% and 30% in the male groups and 0%, 10%, 10%, 0% and 10% in the female groups, in order of ascending dose level. All deaths occurred within 24 hours of treatment, starting 137 minutes post-treatment. There were no deaths or clinical signs in the vehicle control animals. All surviving animals gained weight during the observation period. There were no treatment-related gross lesions at necropsy, and all gross and histological lesions were considered to be incidental to treatment with the 8,9-*Z* isomer of avermectin B_{1a}. The LD_{50} value was estimated to be > 80 mg/kg bw for animals of both sexes, without a significant difference between the sexes.

[d] This was an exploratory acute oral toxicity study using dose levels of 50, 90, 162, 292 and 525 mg/kg bw. All female CD-1 mice treated at 292 and 525 mg/kg bw died within 2 days of treatment, but there were no deaths in the groups treated at 50, 90 or 162 mg/kg bw. Prior to death, the animals treated at 292 and 525 mg/kg bw were moribund within 5 hours of treatment and showed decreased activity, bradypnoea and, in some animals, tremors on handling. Urine staining was also evident in animals treated at 525 mg/kg bw. Animals treated at 162 mg/kg bw showed decreased activity and bradypnoea from day 2 to day 3 and hunched posture on day 3. Animals treated at 90 mg/kg bw showed decreased activity and unkemptness on day 2 only. No clinical signs occurred at 50 mg/kg bw.

[e] This was an exploratory acute oral toxicity study using dose levels of 10, 20 and 30 mg/kg bw. There were one, three and two deaths in male CF-1 mice, in order of ascending dose level. Clinical signs of decreased activity and bradypnoea at all dose levels and ptosis at 30 mg/kg bw occurred within 30 minutes of treatment. Decedents were moribund within 3 hours of treatment, and some animals treated at 20 or 30 mg/kg bw showed tremors on handling. Urine staining was evident at 30 mg/kg bw. Survivors were of normal appearance by day 2.

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 postpartum.

Postmortem examination of F_1 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 postpartum. Litter data, maternal body weights, time to mating and duration of gestation were analysed statistically.

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 feed 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. There were no treatment-related effects on the

external appearance of pups. One pup from the 0.12 mg/kg bw per day group had anencephaly and ethmocephaly at birth, and one pup from the 0.06 mg/kg bw per day group, with a domed head, was found to be severely hydrocephalic at necropsy on day 21. There were no other external malformations. Pup growth, survival and sex ratios were unaffected by treatment at all dose levels. Although postimplantation survival was significantly reduced ($P < 0.05$) in the 0.12 mg/kg bw per 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. As there was no dose–response relationship, the difference is considered to be incidental to treatment. Gross and histomorphological examination of the eyes of F_1 generation progeny revealed no treatment-related effects.

In view of the absence of effects on female fertility and reproductive performance at all doses, a NOAEL for maternal toxicity and reproductive effects in rats for the 8,9-*Z* isomer of avermectin B_{1a} was identified as 0.40 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was also 0.40 mg/kg bw per day, the highest dose tested, based on the absence of effects on external appearance, viability and growth of F_1 generation progeny at all doses.

The study was GLP compliant, and a QA statement was attached (Gordon, 1988b).

Developmental toxicity of 8,9-Z-B_{1a}

Seven groups of 7–13 naturally mated female mice (Crl:CF1 (BR) strain, 82 days old, weight range 23.2–30.9 g) were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with the 8,9-*Z* isomer of avermectin B_{1a} (batch no. L-652,280-00N03, purity 99%) in sesame oil at a dose level of 0 (vehicle only), 1.5, 3.0, 6.25, 12.5, 25.0 or 50.0 mg/kg bw per day from day 6 to day 15 of gestation (day 0 = day on which vaginal plug was observed). Treatment at dose levels of 3.0 mg/kg bw per day and higher was discontinued after a single dose due to mortality. Clinical signs were recorded daily immediately after dosing and also 1–5 hours after dosing on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, and 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. Maternal body weight data and litter parameters were analysed statistically.

There were two treatment-related deaths in each of the groups treated at 3.0, 12.5, 25.0 and 50.0 mg/kg bw per day and three deaths in the group treated at 6.25 mg/kg bw per day after one dose. The animals in these groups were killed and discarded on days 6–8 of gestation. A single treatment-related death also occurred at 1.5 mg/kg bw per day. Death occurred on day 8 of gestation after two doses. There were no other deaths during the study. There were no clinical signs of toxicity other than in some animals that died after one dose that were comatose or moribund prior to death. The overall weight gain during the treatment period at 1.5 mg/kg bw per day was 9.2% lower than in the controls, but the difference was not statistically significant ($P > 0.05$). No gross lesions were evident at necropsy in maternal animals treated at 1.5 mg/kg bw per day. A treatment-induced increase in the incidence of external malformations was apparent at 1.5 mg/kg bw per day. A total of 24 fetuses (28.9%) from four litters had cleft palate, and a further fetus from a litter affected by cleft palate had exencephaly and omphalocele. The overall external malformation incidence of the group treated at 1.5 mg/kg bw per day was 30.1% compared with a control incidence of 0.0%.

In view of the above, the 8,9-*Z* isomer of avermectin B_{1a} elicits both maternal toxicity and a teratogenic response at 1.5 mg/kg bw per day. NOAELs for maternal and fetal toxicity could not be identified, as a treatment-related maternal death and an excess incidence of cleft palates occurred at the lowest dose level tested.

The study was GLP compliant, and a QA statement was attached (Gordon, 1986d).

In a subsequent study, five groups of 12 naturally mated female mice (Crl:CF1 (BR) strain, 10 weeks old, weight range 21.8–27.1 g) were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with the 8,9-*Z* isomer of avermectin B_{1a} (batch no. L-652,280-00N03, purity 99%) in sesame oil at a dose level of 0 (vehicle only), 0.05, 0.1, 0.5 or 1.0 mg/kg bw per day from day 6 to day 15 of gestation (day 0 = day on which vaginal plug was observed). Treatment at dose levels of 3.0 mg/kg bw per day and higher was discontinued after a single dose due to mortality. Clinical signs were recorded daily immediately after dosing and also 1–5 hours after dosing on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, and 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. Maternal body weight data and litter parameters were analysed statistically.

Two deaths (one at 1.0 mg/kg bw per day and the other at 0.5 mg/kg bw per day) occurred during the study, both of which are considered to be treatment related. A female treated at 1.0 mg/kg bw per day was found dead on day 10 after receiving four doses. Clinical signs prior to death were restricted to lethargy and weight loss. One female treated at 0.5 mg/kg bw per day was killed in poor condition on day 11 of gestation. Marked weight loss occurred from the start of treatment, and tremors were evident for 2 days before sacrifice. No other deaths or clinical signs of toxicity occurred at any dose level. Other than in the two animals that died or were sacrificed prematurely, maternal body weight gain was unaffected by treatment at all dose levels. However, the mean weight gains from day 6 to day 17 of gestation in the groups treated at 0.05 and 1.0 mg/kg bw per day were significantly ($P < 0.05$) lower than in the control group (by 18.4%), owing to significantly lower litter sizes. There were no treatment-related gross changes at necropsy in maternal animals of all treatment groups. The mean number of implantations per female in the group treated at 1.0 mg/kg bw per day (9.8) was significantly lower ($P < 0.05$) than the control value of 12.5. Fewer implantations per female also occurred in the group treated at 0.05 mg/kg bw per day. These differences from the controls are considered not to be treatment induced, as implantation was essentially complete at the start of treatment on day 6 of gestation. The incidence of resorptions was higher than the control incidence in all treated groups, but the differences were not statistically significant ($P > 0.05$), and their magnitude did not increase with dose. As a consequence of lower implantation numbers and higher incidences of resorption, the mean number of live fetuses per female was significantly reduced ($P < 0.05$) in the groups treated at 0.05 and 1.0 mg/kg bw per day (8.7 and 8.3, respectively, compared with 11.3 in the controls). As both contributing factors are considered not to be treatment induced, the reduced live litter size is considered not to be related to treatment. Mean fetal weights were unaffected by treatment at all dose levels.

Increased incidences of exencephaly and cleft palate occurred at dose levels of 0.1 mg/kg bw per day and higher, but there was no correlation between incidence and dose level. Cleft palate occurred at incidences between 1.10% (one fetus) and 11.3% (13 fetuses from two litters), compared with a control incidence of 0%. Exencephaly occurred at incidences between 1.74% (two fetuses from one litter) and 4.40% (four fetuses from three litters), compared with a single occurrence (0.73%) in the control group. The similarity of malformations occurring in the treated groups indicated treatment-related teratogenicity at dose levels of 0.1 mg/kg bw per day and higher. Cleft palate and exencephaly did not occur at 0.05 mg/kg bw per day. Two other fetuses from treated litters showed external malformations, one fetus with a malformed tail (1.0 mg/kg bw per day) and one fetus with polydactyly (0.05 mg/kg bw per day). These malformations are not considered to be treatment related.

In view of the above, a NOAEL for maternal toxicity for the 8,9-*Z* isomer of avermectin B_{1a} in the mouse was identified as 0.1 mg/kg bw per day, based on the occurrence of maternal death at levels of 0.5 mg/kg bw per day and higher. The 8,9-*Z* isomer of avermectin B_{1a} is teratogenic in the CF-1 mouse at dose levels of 0.1 mg/kg bw per day and higher, based on excess incidences of cleft palate and exencephaly. A NOAEL for embryo and fetal toxicity was identified as 0.05 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Gordon, 1986e).

In another study, four groups of 25 naturally mated female mice (Crl:CF1 (BR) strain, 10 weeks old, 19.2–28.7 g) were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with 8,9-*Z*-B_{1a} (batch no. L-652,280-00N03, purity 99%) in sesame oil at a dose level of 0 (vehicle only), 0.015, 0.03 or 0.06 mg/kg bw per day from day 6 to day 15 of gestation (day 0 of gestation = day on which vaginal plug was observed). Clinical signs were recorded daily immediately after dosing and also 1–5 hours after dosing on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation. The animals were killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, and implantations were counted and classified as resorptions, dead fetuses or live fetuses. All fetuses were examined externally, weighed and sexed. Visceral examination by dissection was performed on every third fetus of each litter and on all externally abnormal fetuses. The head of every third fetus was fixed in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal malformations and variations. Maternal body weight data and litter parameters were analysed statistically.

There were no deaths or treatment-related clinical signs at any dose level. However, one female treated at 0.06 mg/kg bw per day had only metrial glands at the implantation sites at necropsy, and the animal is considered to have aborted between days 10 and 12 of gestation, when its body weight decreased by 1.7 g. Body weight gain was unaffected by treatment at all dose levels. Litter parameters were unaffected by treatment at all dose levels, and embryonic survival, the proportion of resorbed or dead fetuses and fetal weights were not significantly different ($P > 0.05$) from the control values. There were no treatment-related effects on the incidences of fetal abnormalities or variations at any dose level. However, the overall incidences of abnormal fetuses in all groups treated with 8,9-*Z*-B_{1a} (4.27%, 3.46% and 2.36%, in order of ascending dose) were higher than the control incidence of 1.50%. The difference is considered not to be an effect of treatment, as no dose–response relationship was apparent, and the incidences of individual malformations are considered to reflect spontaneous variation. Three fetuses in each of the groups treated at 0.03 and 0.06 mg/kg bw per day had exencephaly, in three and two litters, respectively. Although the incidences of 1.30% and 1.42%, respectively, are higher than the concurrent control group (0%) and the historical control group (0.27%) incidences, exencephaly has occurred in individual historical control groups at up to 1.64% of fetuses from 14.3% of litters. Furthermore, there is no dose–response relationship for the occurrence of exencephaly, as the incidence of the malformation in the study by Gordon (1986a) was 1.20% (one fetus out of seven litters) at a dose level of 1.5 mg/kg bw per day. A single fetus in the group treated at 0.015 mg/kg bw per day showed cleft palate, which is considered to be a spontaneous occurrence in the absence of cleft palate at higher dose levels. One litter each at 0.015 and 0.03 mg/kg bw per day contained fetuses with hindlimb extension (four and one fetuses, respectively), but the malformation also occurred in a control fetus.

Sternebral, vertebral and rib malformations occurred at low incidence in the treated groups, but none was considered treatment induced, as they also occurred spontaneously in historical control groups. The incidences of visceral and skeletal variations, including the incidences of fetuses with incomplete ossification, were comparable with those of the controls, although sternebral and vertebral sites occurred at slightly higher incidences than in controls.

The NOAEL for both maternal and embryo/fetal toxicity, including teratogenicity, was 0.06 mg/kg bw per day, the highest dose tested, for the 8,9-*Z* isomer of avermectin B_{1a} in the CF-1 mouse.

The study was GLP compliant, and a QA statement was attached (Gordon, 1986f).

In another study, five groups of 25 naturally mated female mice (Crl:CF1 (BR) strain, 9.5 weeks old, 21.5–28.5 g) were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with 8,9-*Z*-B_{1a} (batch no. L-652,280-00N03, purity 99%) in sesame oil at a dose level of 0 (vehicle only), 0.015, 0.03, 0.1 or 0.5 mg/kg bw per day from day 6 to day 15 of gestation (day 0 of gestation = day on which vaginal plug was observed). Clinical signs were recorded daily immediately after dosing and also 1–5 hours after dosing on weekdays. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 17 of gestation, and feed consumption was measured periodically. The animals were

killed on day 17 of gestation and subjected to gross necropsy examination. The uterus was examined to determine pregnancy status, and implantations were counted and classified as resorptions, dead fetuses or live fetuses. All fetuses were examined externally, weighed and sexed. Visceral examination by dissection was performed on every third fetus of each litter and on all externally abnormal fetuses. The head of every third fetus was fixed in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal malformations and variations. Maternal body weight changes and litter parameters (implantations per female, resorptions plus dead fetuses per implantation site, live fetuses per female and live fetal weight) were analysed statistically.

One animal treated at 0.5 mg/kg bw per day was killed in a moribund condition after receiving six doses. The animal had marked weight loss, anorexia, lethargy and chromodacryorrhoea prior to death. No other deaths or treatment-related clinical signs occurred during the study. Maternal body weight and feed consumption were unaffected by treatment at all dose levels, and there were no treatment-related gross changes at necropsy. Litter parameters were unaffected by treatment at all dose levels, and embryonic survival and fetal weights were not significantly different ($P > 0.05$) from the control values. The proportion of resorbed or dead fetuses per implantation in the group treated at 0.03 mg/kg bw per day (16.3%) was significantly ($P < 0.05$) higher than the control value (9.0%). The difference is considered not to be treatment induced, as there was no effect on the parameter in the group treated at 0.1 mg/kg bw per day (9.3% resorbed or dead fetuses per implantation). Thus, no dose–response relationship was evident.

The higher proportion of resorbed or dead fetuses per implantation in the group treated at 0.5 mg/kg bw per day (14.3%) was not statistically significant ($P > 0.05$). The overall incidences of malformed fetuses were 3.86%, 3.78%, 3.23% and 12.4%, in order of ascending dose, compared with a control incidence of 2.30%. An increased incidence of cleft palate occurred in the group treated at 0.5 mg/kg bw per day (24 fetuses in six litters – 10.3%) compared with the control group incidence of 0%, indicating treatment-related teratogenicity. Six fetuses from a single litter treated at 0.1 mg/kg bw per day also had cleft palate. As the group fetal incidence of 2.15% is in excess of the historical control mean incidence (0.28%) and the concurrent control incidence, it is considered to be an effect of treatment. The occurrence of single fetuses with cleft palate in the groups treated at 0.015 and 0.03 mg/kg bw per day is considered not to be treatment induced, as the incidences (0.35% and 0.42%, respectively) are similar to the historical control incidence. Exencephaly occurred at slightly higher incidences in the groups treated at 0.015, 0.03 and 0.5 mg/kg bw per day (0.70%, 2.09% and 0.85%, respectively) compared with the control incidence (0.38%), but the malformation did not occur at 0.1 mg/kg bw per day. As there was no statistically significant ($P > 0.05$) dose–response relationship, the higher incidence at 0.5 mg/kg bw per day is considered not to be treatment related. All other external malformations, open eyelid, micrognathia, cleft lip and tail malformation, occurred in isolation and are considered unrelated to treatment. The incidences of visceral and skeletal malformations did not indicate an effect of treatment at any dose level. At the two highest dose levels, 0.1 and 0.5 mg/kg bw per day, all but one malformation (two fetuses with sternebral malformation at 0.1 mg/kg bw per day) occurred in single fetuses. Higher numbers of fetuses with sites of incomplete ossification at 0.03 and 0.1 mg/kg bw per day are considered incidental to treatment because the incidence at the highest dose level, 0.5 mg/kg bw per day, was similar to the control incidence.

In view of the above, a NOAEL for maternal toxicity for the 8,9-*Z* isomer of avermectin B_{1a} in CF-1 mice was identified as 0.1 mg/kg bw per day, based on the occurrence of one treatment-related death at 0.5 mg/kg bw per day. A teratogenic effect, characterized by an increased incidence of cleft palate, occurs in response to treatment with the 8,9-*Z* isomer of avermectin B_{1a} at 0.5 mg/kg bw per day. The minimum teratogenic dose level (LOAEL) is 0.1 mg/kg bw per day, based on a slight increase in the incidence of cleft palate. Therefore, a clear NOAEL for embryo/fetal toxicity in CF-1 mice was identified as 0.03 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Gordon, 1986g).

Table 22. Combined data for observation of excencephaly in oral maternal toxicity/teratogenicity studies with the 8,9-Z isomer of avermectin B_{1a} in CF-1 mice

Dose (mg/kg bw per day)	Reference	Litters examined	Litters with exencephaly		Fetuses examined	Fetuses with exencephaly	
			N	%		*N*	%
Control	Gordon (1986d)	13	0	0	164	0	0
	Gordon (1986e)	12	1	8.3	137	1	0.73
	Gordon (1986f)	22	0	0	200	0	0
	Gordon (1986g)	23	1	4.3	261	1	0.38
0.015	Gordon (1986f)	22	0	0	211	0	0
	Gordon (1986g)	24	1	4.2	285	1	0.35
0.03	Gordon (1986f)	23	3	13.0	231	3	1.3
	Gordon (1986g)	23	2	8.6	239	5	2.1
0.05	Gordon (1986e)	12	0	0	104	0	0
0.06	Gordon (1986f)	22	2	9.1	212	3	1.4
0.1	Gordon (1986e)	11	1	9.1	115	2	1.7
	Gordon (1986g)	24	0	0	279	0	0
0.5	Gordon (1986e)	9	3	33.3	91	4	4.3
	Gordon (1986g)	23	1	4.3	234	1	0.43
1.0	Gordon (1986e)	11	1	9.1	91	2	2.3
1.5	Gordon (1986f)	7	1	14.3	83	1	1.2
Historical control	From eight studies between 1984 and 1987		Mean	4.3		Mean	0.7
			Minimum	0		Minimum	0
			Maximum	16.7		Maximum	4.1

The common developmental defects seen in the above developmental toxicity studies were combined and compared with the historical controls (Table 22 and Table 23).

In an oral developmental toxicity study in CD-1 mice, four groups of 22 naturally mated female mice (Crl:CD-1 (BR) strain, 12 weeks old, weight range 23.8–32.4 g) were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with the 8,9-*Z* isomer of avermectin B_{1a} (batch no. L-652,280-000N010, purity 98.1%) in sesame oil at a dose level of 0 (vehicle only), 0.75, 1.5 or 3.0 mg/kg bw per day from day 6 to day 15 of gestation (day 0 of gestation = day on which vaginal plug was observed). Periodic analysis of the formulations demonstrated acceptable achieved concentrations (within 10% of the nominal concentration). Clinical signs were recorded on day 0 of gestation and twice daily from day 6 to day 18 of gestation. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 18 of gestation, and feed 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. Placentas 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 in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal abnormalities and variations.

Table 23. Combined data for observation of cleft palate in oral maternal toxicity/teratogenicity studies with the 8,9-Z isomer of avermectin B_{1a} in CF-1 mice

Dose (mg/kg bw per day)	Reference	Litters examined	Litters with cleft palate		Fetuses examined	Fetuses with cleft palate	
			N	%		*N*	%
Control	Gordon (1986d)	13	0	0	164	0	0
	Gordon (1986e)	12	0	0	137	0	0
	Gordon (1986f)	22	0	0	200	0	0
	Gordon (1986g)	23	0	0	261	0	0
0.015	Gordon (1986f)	22	1	4.5	211	1	0.47
	Gordon (1986g)	24	1	4.2	285	1	0.35
0.03	Gordon (1986f)	23	0	0	231	0	0
	Gordon (1986g)	23	1	4.3	239	1	0.42
0.05	Gordon (1986e)	12	0	0	104	0	0
0.06	Gordon (1986f)	22	0	0	212	0	0
0.1	Gordon (1986e)	11	2	18.2	115	13	11.3
	Gordon (1986g)	24	1	4.2	279	6	2.2
0.5	Gordon (1986e)	9	1	11.1	91	1	1.1
	Gordon (1986g)	23	6	26.1	234	24	10.3
1.0	Gordon (1986e)	11	4	36.4	91	7	7.7
1.5	Gordon (1986g)	7	4	57.1	83	24	28.9
Historical control	From 11 studies between 1983 and 1987		Mean	2.9		Mean	0.3
			Minimum	0		Minimum	0
			Maximum	14.3		Maximum	1.3

bw: body weight

There were no deaths and no treatment-related clinical signs during the study. There were no treatment-related effects on maternal body weight gain or feed consumption. There were no treatment-related gross findings at necropsy in maternal animals. Pregnancy indices, embryo survival parameters and fetal weights were unaffected by treatment. There were no treatment-related external, visceral or skeletal alterations in fetuses. Minor group differences in the low incidence of cleft palate showed no dose–response relationship and were well within the historical control range for CD-1 mice. There was a slight increase in the incidence of postaxial pseudopolydactyly in the 3.0 mg/kg bw per day group: 11 fetuses (3.5%), compared with two fetuses (0.7%) in the concurrent control group and none in the historical control group. However, the increased incidence of this minor alteration was not considered to be treatment related, as the increase was restricted to mainly one litter, which had nine of the 11 fetuses with the alteration.

In view of the above, it is concluded that there was no evidence of maternal toxicity or embryo/fetal toxicity up to 3.0 mg/kg bw per day when the 8,9-*Z* isomer of avermectin B_{1a} was administered to CD-1 mice. Hence, the NOAELs for both maternal and embryo/fetal toxicity were 3.0 mg/kg bw per day, the highest dose tested.

The study was GLP compliant, and a QA statement was attached (Wise, 1996a).

Developmental toxicity of 8,9-Z-B_{1a} in a pregnant subpopulation of mice sensitive and insensitive to the tremor-inducing properties of abamectin

This study was undertaken to ascertain the developmental toxicity of the 8,9-*Z* isomer of avermectin B_{1a} to a pregnant subpopulation of CF-1 mice sensitive or insensitive to the tremor-inducing properties of abamectin technical. Subpopulations of mice sensitive and insensitive to the tremor-inducing properties of abamectin were identified. Initially, 276 female mice (Crl:CF1 (BR) strain, 9–10 weeks old, 21.7–32.3 g) were treated with a single gavage dose of abamectin technical (batch no. L-676,863-000V098, purity 97.1%) at 0.4 mg/kg bw in sesame oil. The animals were observed for clinical signs approximately hourly on the day of treatment, daily for 3 days thereafter and then twice weekly until mating. Following preliminary identification of insensitive individuals, the subpopulation was treated with a further oral abamectin dose of 0.8 mg/kg bw in sesame oil to confirm its insensitive status. The insensitive animals and the surviving sensitive animals were naturally mated 2–3 weeks later for a developmental toxicity study. All animals that died or were killed were discarded without necropsy.

Animals selected for the developmental toxicity study were maintained for 2–3 weeks prior to mating. Four groups of 25 naturally mated insensitive female mice were treated orally, by gavage, at a constant treatment volume of 10 mL/kg bw with the 8,9-*Z* isomer of avermectin B_{1a} (batch no. L-652,280-000N007, purity 79.8%) in sesame oil at a dose level of 0 (vehicle only), 0.5, 1.0 or 1.5 mg/kg bw per day from day 6 to day 15 of gestation (day 0 of gestation = day on which vaginal plug was observed). Two further groups of sensitive mice were similarly treated with 0 (four animals) or 0.2–1.0 mg/kg bw per day (18 animals). (The dose level was increased to 0.3 mg/kg bw per day after 1–3 doses. Two days later, the dose level was increased to 0.5 mg/kg bw per day. One day later, the dose level was increased to 1.0 mg/kg bw per day for 1 day. Dosing was then suspended for 2 days and recommenced at 0.75 mg/kg bw per day in 6/18 females of normal appearance. Twelve animals with adverse clinical signs were killed, examined for pregnancy status and discarded.) Periodic analysis of the formulations demonstrated acceptable achieved concentrations (within 10% of the nominal concentration). Clinical signs were recorded on day 0 of gestation and twice daily from day 6 to day 18 of gestation. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16 and 18 of gestation, and feed consumption was measured at 3-day intervals from day 3 of gestation. Surviving animals were killed on day 18 of gestation and subjected to a gross necropsy examination of the thoracic and abdominal cavities. Gross lesions were preserved for possible histological examination. The brain (cerebrum and cerebellum) was removed and frozen in isopentane/liquid nitrogen. The brains of all surviving sensitive animals and of seven insensitive vehicle control animals and 11 insensitive animals treated at 1.5 mg/kg bw per day were processed for P-glycoprotein immunohistochemistry. In addition, one half of each brain from the sensitive animals was also submitted to western immunoblot analysis of P-glycoprotein. Slides were examined microscopically using a blind procedure and scored for relative staining intensity for P-glycoprotein after examining appropriate control sections to evaluate background staining. 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. Placentas 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 in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal abnormalities and variations.

In the preliminary sensitivity testing phase, 27% of animals 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 four were allocated to the vehicle control group and 18 allocated to be treated at 0.2–1.0 mg/kg bw per 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 per 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 per day. Recumbency or decreased activity occurred in all animals after one or two doses at 1.0 mg/kg bw per day.

Four animals survived to day 18, but three were recumbent for at least 2 days before necropsy. Treatment-related effects on maternal body weight gain and feed consumption were confined to the sensitive group treated at 0.2–1.0 mg/kg bw per day. A 74.2% decrease in weight gain occurred from day 6 to day 16, resulting in a group mean body weight of 39.0 g compared with the vehicle control sensitive group value of 53.3 g. Feed consumption was markedly reduced from day 11 of gestation. Decreases of 42.4% and 83.0% occurred on days 11 and 17 of gestation, respectively, compared with the vehicle control sensitive group. The overall group mean feed consumption values of the treated insensitive groups were 6.40, 6.36 and 6.50 g/day compared with the vehicle control insensitive group value of 6.54 g/day. There were no treatment-related gross changes in maternal animals at any dose level. Three of the four sensitive animals treated at 0.2–1.0 mg/kg bw per day produced dead fetuses only, which is considered to be a consequence of prolonged maternal inactivity. In the insensitive groups, there were no treatment-related effects on litter parameters at any dose level, as assessed by preimplantation and postimplantation losses, mean numbers of corpora lutea, implantations and live fetuses, fetal weights and sex ratios. 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 fetuses, 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 per day. The incidence of the variation postaxial pseudopolydactyly was increased in all treated insensitive groups (8.8–14.7%) compared with a control incidence of 5.1%. This is considered not to be treatment related, as the incidences lie within, or very close to, the historical control range of 5.8–13%. The nature, incidence and distribution of other external malformations and variations and all visceral and skeletal malformations and variations in the treated insensitive groups did not suggest an effect of treatment. 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.

In view of the above, the developmental toxicity of the 8,9-*Z* isomer of avermectin B_{1a} to a subpopulation of CF-1 mice sensitive to the tremor-inducing properties of abamectin could not be evaluated, as only a single viable litter was produced. However, developmental toxicity, characterized 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 1.5 mg/kg bw per day. Therefore, a NOAEL for embryo and fetal toxicity could not be established. The isomer did not produce maternal toxicity at dose levels up to 1.5 mg/kg bw per day.

The study was GLP compliant, and a QA statement was attached (Wise, 1996b).

Exploratory study of oral developmental toxicity of 8,9-Z-B_{1a}

To undertake an exploratory study of the oral developmental toxicity of the 8,9-*Z* photoisomer in CF-1 mice of known P-glycoprotein genotype, female mice of known genotype for the *mdr1* gene encoding the P-glycoproteins and mated to male mice of known genotype were used. Prior to initiation of the study, male and female mice (strain Crl:CF-1 (BR)) were submitted for restriction fragment length polymorphism Southern blot analysis to determine the genotype for the *mdr1* gene. The females (11 weeks old, body weight range 22.8–30.7 g) were mated with sexually mature males to provide groups of 12 mated females producing fetuses of genotypes predictable by the principles of Mendelian inheritance.

The five groups of 12 mated females were treated orally, by gavage, with the 8,9-*Z* photoisomer of avermectin B_{1a} (batch no. L-652,280-000N009, purity 94.3%) in sesame oil at a dose level of 0 (vehicle only) or 1.5 mg/kg bw per day from day 6 to day 15 of gestation (day 0 of gestation = day on which vaginal plug was observed). Clinical signs were recorded daily on day 0 of gestation and twice daily from day 6 to day 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 was examined to determine pregnancy status. A distal tail segment was retained from each maternal animal for possible reanalysis of P-glycoprotein genotype. Implantations were counted and classified as resorptions, live fetuses or dead fetuses. All fetuses were weighed and examined for external malformations, including the palate. One hindlimb of each fetus was removed for possible analysis of P-glycoprotein genotype. At least four litters per group were genotyped using DNA isolated from the hindlimb. Placentas from one vehicle control (–/–), one 1.5 mg/kg bw per day female (+/+) and two vehicle controls (+/–) were retained for western blotting biochemical analysis of P-glycoprotein. The heads and placentas of fetuses from four vehicle control (+/–) females and four females treated at 1.5 mg/kg bw per day (+/– × –/–) were frozen in isopentane/liquid nitrogen for P-glycoprotein immunohistochemistry of brain endothelium, palate epithelium, placental trophoblasts and placental yolk sac endothelium. The frozen sections were analysed immunohistochemically for P-glycoprotein using an adaptation of an external reference (Schinkel et al., 1994). Slides were examined microscopically using a blind procedure and scored for relative staining intensity for P-glycoprotein compared with control sections. The brain of one female treated at 1.5 mg/kg bw per day (+/+) was retained for possible immunohistochemical analysis.

No deaths or treatment-related clinical signs or body weight effects occurred during the study. The weight gain from day 6 to day 18 of the vehicle control group (+/– × +/–) was 12.4–20.8% higher than in all other study groups, but all 8,9-*Z* isomer–treated groups showed weight gains comparable with those of the second vehicle control (–/– × –/–) group. There were no treatment-related effects on embryo survival or litter parameters in any treated group, as assessed by mean number of implantation sites, postimplantation loss, mean number of live fetuses per litter, fetal weight and sex ratio. There were increased incidences of cleft palate in two of the three groups treated at 1.5 mg/kg bw per day. The +/– female × +/+ male group produced 18 fetuses with cleft palate (litter mean incidence 12.0%), and the +/– female × –/– male group produced 80 fetuses with cleft palate (litter mean incidence 58.0%), compared with litter mean incidences of 0.83% and 0% in the vehicle control groups (+/– × +/– and –/– × –/–, respectively). The incidence of cleft palate was 0% in the homozygous positive group (+/+ × +/+) treated at 1.5 mg/kg bw per day. The incidence of postaxial pseudopolydactyly was higher in the +/– × –/– group (litter mean incidence 19.0%) compared with incidences in the vehicle control groups of 2.8% and 8.3%. The difference is considered incidental to treatment, as the high litter mean incidence was due primarily to a single affected fetus. All other observed abnormalities occurred in isolation and are considered unrelated to treatment with 8,9-*Z*-B_{1a}.

Genotyping of fetuses from at least four litters per 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 fetuses derived from treated females in either of the groups in which the genotype occurred had cleft palate, whereas 30/31 (97%) –/– genotype fetuses exposed to 8,9-*Z*-B_{1a} had cleft palate, and 29/70 (41%) of the +/– genotype fetuses were affected.

Histopathological evaluation of fetal brain revealed diffuse P-glycoprotein staining on the endothelial cell surface of cerebral and cerebellar capillaries in most of the heterozygous (+/–) and homozygous positive (+/+) fetuses. 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 fetuses 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 fetuses in the study. Western blotting analysis of placentas for *mdr1a* P-glycoprotein confirmed the results of the immunohistochemical analysis; the amount of P-glycoprotein in the placenta varies with fetal genotype. Thus, the highest

amounts were detected in the +/+ placentas, with lesser amounts in the +/– placentas, and P-glycoprotein was absent in –/– placentas.

In view of the above, the dose level of 1.5 mg/kg bw per day of the 8,9-*Z* isomer of avermectin B_{1a} was identified as the NOAEL for maternal toxicity and reproductive indices, but elicited embryotoxicity, manifested as increased incidences of cleft palate in sensitive fetuses. Fetal sensitivity to the induction of cleft palate is influenced by genotype for the *mdr1* gene encoding for the P-glycoprotein, a gene governed by Mendelian inheritance. Fetal genotype for the *mdr1* 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 with the incidence of cleft palate.

The study was GLP compliant, and a QA statement was attached (Lankas, 1996a,b).

Developmental toxicity of 8,9-Z-B_{1a} in rats

Four groups of 25 naturally mated female rats (Crl:CD(SD) BR strain, approximately 2.5 months old, 201–269 g) were treated orally, by gavage, with the 8,9-*Z* isomer of avermectin B_{1a} (batch no. L-652,280-000N005, purity 91.6%) in sesame oil at a dose level of 0 (vehicle only), 0.25, 0.5 or 1.0 mg/kg bw per day from day 6 to day 17 of gestation (day 0 of gestation = day on which plug or sperm in vaginal smear were observed). The animals were observed for clinical signs daily from day 6 to day 20 of gestation and also 1–5 hours after dosing during the treatment period. Body weights were recorded on days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation. The feed consumption of all animals was measured at 3-day intervals from day 3 to day 20 of gestation. The animals were killed on day 20 and subjected to a gross postmortem examination. Maternal gross lesions were preserved and subsequently examined microscopically. The pregnancy status was established, and the number of corpora lutea was determined. The uterine horns were examined, the number of implantation sites was enumerated and the implantations were classified as live fetuses, dead fetuses or resorptions. The fetuses were removed from the uterine horns and individually weighed, sexed and examined for external malformations. Visceral examination by dissection was performed on every third fetus of each litter and on all externally abnormal fetuses. The head of every third fetus was fixed in Bouin's fluid for subsequent examination by free-hand sectioning. All fetuses were fixed, cleared, stained with alizarin red and examined for skeletal malformations and variations. Litter data (preimplantation loss, implantation number, postimplantation loss, litter size and fetal weight) and maternal body weight data were analysed statistically.

There were no deaths and no treatment-related clinical signs during the study. Females treated at 0.5 and 1.0 mg/kg bw per day showed a treatment-related enhanced body weight gain during the treatment period. The mean weight gains of these groups were 8.9% higher than in the control group and statistically significant ($P < 0.05$). There was no effect on weight gain in the group treated at 0.25 mg/kg bw per day. Feed consumption was unaffected by treatment at all dose levels. There were no treatment-related gross changes at necropsy in maternal animals, and pregnancy incidences were comparable in all groups. There were no treatment-related effects at any dose level on preimplantation and postimplantation losses, live litter size, sex ratio or fetal weights. Preimplantation loss in the group treated at 0.25 mg/kg bw per day (13.4%) was significantly higher ($P < 0.05$) than the control value (7.9%), but because there was no dose–response relationship and because implantation is essentially complete at the commencement of dosing, it is considered to be incidental to treatment. There was no evidence of developmental toxicity, either embryonic/fetal growth retardation or teratogenicity, at any dose level, based on the incidences of external, visceral and skeletal malformations, variations and unossified centres. The overall incidences of malformed fetuses in the treated groups were 1.81%, 3.21% and 2.56%, in order of ascending dose level, compared with a control incidence of 1.69%. The fetal malformations observed in treated groups, clubbed forefoot, hindlimb rotation, situs inversus, missing vertebrae and hypoplastic rib, either were single occurrences or occurred at an incidence comparable with that of the control group. The incidences of fetal variations and sites of incomplete ossification were comparable with those of the control group.

In view of the above, the NOAEL for maternal effects and embryo/fetal toxicity, including teratogenicity, was identified as 1.0 mg/kg bw per day, the highest dose tested, for the 8,9-*Z* isomer of avermectin B_{1a}, based on an absence of adverse effects at all doses.

The study was GLP compliant, and a QA statement was attached (Gordon, 1988a).

3. Observations in humans

Manufacturing employees are medically examined by company physicians at the beginning of their employment and then routinely once a year. Abamectin was manufactured at the Syngenta (formerly Novartis) formulation site at Monthey, Switzerland, from 1998 to 2000 and at the Syngenta (formerly Novartis) formulation site at Rezende, Brazil, from 1999 to 2000, with annual production volumes (formulation) in the range of 90–900 tonnes. Questionnaires have also been completed by the responsible heads of formulation sites and company physicians. No adverse health effects have been reported that could be related to abamectin (Syngenta, 2015).

Human intoxication with abamectin is uncommon. However, there are a number of published reports regarding acute poisoning due to abamectin, mainly ingested for suicidal purposes. Data on humans related to poisoning are consistent with studies on experimental animals and do not demonstrate high acute toxicity. Humans show a low susceptibility towards the toxicity of abamectin. However, depending on the dose consumed, there are variable signs and symptoms, basically due to effects on the nervous system. These include altered mental status, drowsiness, hypotension, tachycardia and dermal erythema (Aminiahidashti, Jamali & Gorji, 2014); mild poisonings led mainly to mild gastrointestinal symptoms (nausea, vomiting and diarrhoea) or short-lasting central nervous system depression (dizziness, drowsiness and weakness) (Chung, 1999); tremor and vomiting (Soyuncu et al., 2007); nystagmus, which may be affected due to the vestibular cerebellar system (Wang et al., 2013); neurological toxicity and respiratory failure (Bansod et al., 2013); and acute onset of confusion, blurred vision and unsteady gait, restlessness and agitation, and partial ptosis of both eyes (Karunatilake et al., 2012). The therapy for abamectin poisoning is mainly symptomatic and supportive. Despite the lack of specific therapy, the follow-up of severely poisoned patients showed an uneventful recovery. Therefore, the prognosis is good, unless complications occur from severe uncontrolled aspiration.

No epidemiological studies on abamectin were identified in the literature.

Comments

Susceptibility of CF-1 mice and neonatal rats to abamectin

In mammals, abamectin is a substrate for P-glycoprotein, which is a member of the adenosine triphosphate (ATP)–binding cassette subfamily B (ABCB1), also known as multidrug resistance protein 1 (MDR1). P-glycoprotein is extensively expressed in the intestinal epithelium, liver cells, cells of the proximal tubule of the kidney and capillary endothelial cells of the brain (blood–brain barrier), placenta, ovaries and testes. As an efflux transporter, P-glycoprotein acts as a protective barrier to keep xenobiotics out of the body by excreting them into bile, urine and intestinal lumen and prevents the accumulation of these compounds in the brain and gonads, as well as the fetus. Therefore, some test animals (e.g. CF-1 mice) in which genetic polymorphisms compromise P-glycoprotein expression are particularly susceptible to abamectin-induced toxicity. As a result, studies on CF-1 mice were not considered relevant for the human risk assessment.

In rats, P-glycoprotein was undetectable in the embryo and early stages of postnatal development. In the brush border of intestinal epithelial cells, expression of P-glycoprotein was absent to minimal on postnatal day 8 and was more intense but did not reach adult levels by postnatal day 20 (Cukierski, 1995; Lankas, 1996a). In the brain capillaries, expression of P-glycoprotein was minimal in fetuses on gestation day 20 and in younger pups until postnatal day 11, with subsequent increases to a plateau at adult levels by postnatal days 20–28 (Cukierski, 1995; Lankas, 1996a; Matsuoka et al.,

1999). In humans, P-glycoprotein expression in fetal intestinal epithelium and brain capillaries is at the adult level at week 28 of gestation (van Kalken et al., 1992), and therefore results of reproductive toxicity studies in neonatal rats should be interpreted accordingly.

Biochemical aspects

In rats, orally administered radiolabelled avermectin B_{1a} was rapidly and almost completely absorbed, based on a comparison with urinary excretion after intravenous administration. Maximum concentrations in blood were achieved within 4–8 hours after administration. Radiolabel was widely distributed. Elimination of radiolabel occurred predominantly by non-biliary excretion into the gastrointestinal tract and excretion in the faeces, whereas urinary excretion accounted for only 0.5–1.4% of the dose. Elimination accounted for 80–101% of the administered dose within 96 hours. Rate of oral absorption, tissue distribution and excretion were independent of the dose level, treatment regimen and/or sex; however, the depletion of tissue residues was approximately 2-fold more rapid in males than in females. There was no evidence for tissue accumulation on repeated administration (Alvaro et al., 1984; Hassler, 2001, 2003; Briswalter, 2003a).

In toxicokinetic studies in genotyped subpopulations of CF-1 mice (i.e. *mdr1a* P-glycoprotein–deficient mice and mice with normal expression of *mdr1a* P-glycoprotein), plasma levels of avermectin B_{1a} were higher in *mdr1a* P-glycoprotein–deficient mice than in mice with normal expression of *mdr1a* P-glycoprotein. Distribution and elimination appeared to be slower in *mdr1a* P-glycoprotein–deficient mice, with concentrations in the brain tissue of *mdr1a* P-glycoprotein–deficient mice up to 160 times higher than in mice with normal expression of *mdr1a* P-glycoprotein. The primary route of excretion was via faeces (Gledhill, 2008).

Metabolism of avermectin B_{1a} in the rat was moderate to extensive and proceeded predominantly via demethylation, hydroxylation, cleavage of the oleandrosyl ring and oxidation reactions. The metabolite pattern in urine, faeces and bile was complex but qualitatively independent of the sex and dose, with some quantitative variations. Eleven metabolites were isolated. Unchanged avermectin B_{1a} and the metabolites 3″-*O*-desmethyl-avermectin B_{1a} (3″DM), 24a-hydroxy-avermectin B_{1a} (24aOH), 27-hydroxy-avermectin B_{1a} (27OH), 3″-*O*-desmethyl, 24a-hydroxy-avermectin B_{1a} (3″DM,24aOH) and 3″-*O*-desmethyl, 27-hydroxy-avermectin B_{1a} (3″DM,27OH) represented the majority of the faecal radioactivity (Alvaro et al., 1984; Hassler, 2003).

Toxicological data

Abamectin was of high acute oral toxicity, with an LD_{50} of 8.7 mg/kg bw in rats with sesame oil as the vehicle (Robertson, 1981a) and an LD_{50} of 214 mg/kg bw in rats with water as the vehicle (Glaza, 2001). The acute inhalation toxicity of abamectin was also high, with an LC_{50} of > 0.034 and < 0.21 mg/L in rats (Ruddock, 2001a; Noakes, 2003). The dermal LD_{50} in both rats and rabbits was greater than 2000 mg/kg bw (Gordon, 1984a; Sieber, 2011). Abamectin was not irritating to the eye (Glaza, 2000) or the skin of rabbits (Robertson, 1981b). It was not a dermal sensitizer in guinea-pigs (Ruddock, 2001b).

In short- and long-term toxicity studies performed in rats and dogs, clinical signs observed as a response to treatment were tremors in rats and dogs and mydriasis or absent pupil reflex in dogs. There were no histopathological changes in the tissues of the central and peripheral nervous systems. The clinical signs are considered to be an exaggerated pharmacological response to the interaction of abamectin with the gamma-aminobutyric acid (GABA)–benzodiazepine receptor chloride channel complex. Treatment-related histopathological alterations in dogs were confined to the hepatobiliary system. Based on the severity of clinical signs of neurotoxicity and mydriasis and the doses at which death occurs, the dog was more sensitive than the rat to abamectin.

In an 8-week dietary toxicity study in rats with mean achieved dose levels of 0, 0.5, 1.2, 1.9, 2.2 and 5.0 mg/kg bw per day, deaths occurred at 2.2 and 5.0 mg/kg bw per day. The NOAEL was 1.2 mg/kg bw per day, based on the occurrence of clinical signs and reduced body weight gain at 1.9 mg/kg bw per day and above (Gordon, 1984b). No 90-day toxicity study in rats was provided.

In 12-week, 18-week and 53-week toxicity studies in dogs, a very steep dose–response curve for abamectin was noted. In the 12-week feeding study in dogs with dietary concentrations of abamectin adjusted to provide dose levels of 0, 0.25, 0.5, 1.0 and 4.0/2.0 mg/kg bw per day, the NOAEL was 0.5 mg/kg bw per day, based on the occurrence of mydriasis starting in week 1 of treatment at 1.0 mg/kg bw per day. Markedly reduced feed consumption, body weight loss and clinical signs of intoxication were observed at 2.0 mg/kg bw per day and above (Gordon, 1984c).

In the 18-week gavage study in dogs administered abamectin at a dose of 0, 0.25, 0.5, 2.0 or 8.0 mg/kg bw per day, the NOAEL was 0.25 mg/kg bw per day, based on mortality, clinical signs of toxicity, reduced body weight gain or body weight loss, and histopathological changes in the liver at 0.5 mg/kg bw per day. Signs of toxicity were observed starting on day 7 at 0.5 mg/kg bw per day, on day 3 at 2.0 mg/kg bw per day and on day 1 (within 3 hours after dosing) at 8.0 mg/kg bw per day (Allen & Robertson, 1976; Allen, 1982).

In the 53-week feeding study in dogs with dietary concentrations of abamectin adjusted to provide dose levels of 0, 0.25, 0.5 and 1.0 mg/kg bw per day, the NOAEL was 0.25 mg/kg bw per day, based on mydriasis at 0.5 mg/kg bw per day. Signs of toxicity were observed starting in the first week at 0.5 and 1.0 mg/kg bw per day (Gordon, 1984d).

The overall NOAEL for the three dog studies was 0.25 mg/kg bw per day, based on mortality (one animal), clinical signs of toxicity (including mydriasis) and reduced body weight gain or body weight loss at 0.5 mg/kg bw per day.

In a 93-week toxicity and carcinogenicity study in mice, with dietary concentrations of abamectin adjusted to provide dose levels of 0, 2.0, 4.0 and 8.0 mg/kg bw per day, the NOAEL was 4.0 mg/kg bw per day, based on increased mortality in males and reduced body weight gain in females at 8.0 mg/kg bw per day. There was no increase in tumour incidence (Gordon, 1985a).

In a 104-week chronic toxicity and carcinogenicity study in rats, with dietary concentrations of abamectin adjusted to provide dose levels of 0, 0.75, 1.5 and 2.0/2.5 mg/kg bw per day, the NOAEL was 1.5 mg/kg bw per day, based on the occurrence of clinical signs of toxicity at 2.0/2.5 mg/kg bw per day. There was no increase in tumour incidence (Gordon, 1985b).

The Meeting concluded that abamectin is not carcinogenic in mice or rats.

Abamectin has been tested for genotoxicity in an adequate range of assays, both in vitro (Gordon, 1983, 1986a,b; Deparade, 2001) and in vivo (Blazak, 1983). There was no evidence of genotoxicity.

The Meeting concluded that abamectin is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that abamectin is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats, abamectin (in sesame oil) was administered orally by gavage at a dose level of 0, 0.05, 0.12 or 0.40 mg/kg bw per day. The NOAEL for parental toxicity and reproductive toxicity was 0.40 mg/kg bw per day, the highest dose tested. For offspring toxicity, the NOAEL was 0.12 mg/kg bw per day, based on increased pup mortality and retarded weight gain in both F_1 and F_2 generation progeny and an increased incidence of transient retinal folds in the eyes of F_2 generation weanlings at 0.40 mg/kg bw per day (Gordon, 1984e).

In a developmental toxicity study in rats, abamectin (in sesame oil) was administered orally by gavage at a dose level of 0, 0.4, 0.8 or 1.6 mg/kg bw per day. The NOAEL for maternal toxicity and embryo/fetal toxicity was 1.6 mg/kg bw per day, the highest dose tested (Gordon, 1982a).

In a developmental toxicity study in rabbits, abamectin (in sesame oil) was administered orally by gavage at a dose level of 0, 0.5, 1.0 or 2.0 mg/kg bw per day. The NOAEL for maternal toxicity was 1.0 mg/kg bw per day, based on the occurrence of severe maternal toxicity (body weight loss) during gestation at 2.0 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1.0 mg/kg bw per day, based on increased incidences of external malformations (cleft palate,

omphalocele, clubbed forefeet) and incomplete ossification of sternebrae and metacarpals at 2.0 mg/kg bw per day (Gordon, 1982b).

The Meeting concluded that abamectin is teratogenic in rabbits, but not in rats.

In an acute neurotoxicity study in rats, abamectin (in sesame oil) was administered orally by gavage at a dose level of 0, 0.5, 1.5 or 6 mg/kg bw. The NOAEL for acute neurotoxicity was 0.5 mg/kg bw, based on a reduced splay reflex at 1.5 mg/kg bw. This change was seen at 6–7 hours after dosing on day 1 and was consistent with the neurotoxic effects observed at higher doses. No neuropathological changes were observed at dose levels up to 6 mg/kg bw (Brammer, 2006a).

In a 90-day neurotoxicity study in rats, abamectin (in sesame oil) was administered orally by gavage at a dose level of 0, 0.4, 1.6 or 4.0 mg/kg bw per day. The NOAEL for systemic toxicity and neurotoxicity was 1.6 mg/kg bw per day, based on reduced body weights in females, clinical signs in both sexes (irregular breathing, upward curvature of the spine, reduced righting reflex, reduced splay reflex) and changes in hindlimb grip strength in females at 4.0 mg/kg bw per day (Brammer, 2006b).

In a developmental neurotoxicity study in rats, abamectin (in sesame oil) was administered orally by gavage to parental females once daily from day 7 of gestation until day 22 postpartum at a dose level of 0, 0.12, 0.20 or 0.40 mg/kg bw per day. In parental females, body weights and feed consumption were slightly increased at all dose levels. In offspring, lower body weights post-weaning and hence a delay in time of vaginal opening were observed at 0.20 mg/kg bw per day and above. The NOAEL was 0.12 mg/kg bw per day. There was no effect on function or morphology of the nervous system at dose levels up to 0.40 mg/kg bw per day, the highest dose tested (Moxon, 2005).

In a supplementary developmental neurotoxicity study in rats performed to provide brain morphometry data, abamectin (in sesame oil) was administered orally by gavage to parental females once daily from day 7 of gestation until day 22 postpartum at a dose level of 0, 0.12, 0.20 or 0.40 mg/kg bw per day. In parental females, body weights and feed consumption were slightly increased at all dose levels during gestation. In offspring at 0.40 mg/kg bw per day, clinical signs and increased mortality were observed in the pre-weaning period, and therefore all dams and pups in this group were removed from the study on days 15–38 postpartum. At 0.20 mg/kg bw per day, body weights of pups post-weaning were statistically significantly lower and the time of vaginal opening was statistically significantly later compared with the control group. The NOAEL for offspring toxicity was 0.12 mg/kg bw per day. There was no effect on function or morphology of the nervous system at dose levels up to 0.40 mg/kg bw per day, the highest dose tested (Moxon, 2007).

In a gavage study in rhesus monkeys, the most sensitive indicator of abamectin toxicity was emesis, as clinical signs of toxicity seen in mice and rats (tremors and convulsions) did not occur. The minimum toxic dose (LOAEL) of abamectin was 2.0 mg/kg bw, and the NOAEL was 1.0 mg/kg bw (Gordon, 1985c).

The Meeting concluded that abamectin is neurotoxic.

Toxicological data on metabolites and/or degradates

The 8,9-*Z* isomer of avermectin (also referred to as the Δ-8,9-isomer, NOA 427011 and L-652,280) is a photodegraded product of abamectin.

An exploratory acute oral toxicity study with the 8,9-*Z* isomer of avermectin B_{1a} in CD-1 mice and CF-1 mice gave LD_{50} values of 217 and 20 mg/kg bw, respectively (Lynch, 1996).

The 8,9-*Z* isomer of avermectin B_{1a} did not induce gene mutations in bacteria, with or without metabolic activation (Gordon, 1988c).

In a one-generation reproductive toxicity study with the 8,9-*Z* isomer of avermectin B_{1a} in rats using dose levels of 0, 0.06, 0.12 and 0.40 mg/kg bw per day, the NOAEL for maternal toxicity, reproductive effects and effects on offspring was 0.40 mg/kg bw per day, the highest dose tested (Gordon, 1988b).

In a developmental toxicity study with the 8,9-*Z* isomer of avermectin B_{1a} in CD-1 mice using dose levels of 0, 0.75, 1.5 and 3.0 mg/kg bw per day, the NOAEL for maternal effects and embryo/fetal toxicity, including teratogenicity, was 3.0 mg/kg bw per day, the highest dose tested (Wise, 1996a).

In a developmental toxicity study with the 8,9-*Z* isomer of avermectin B_{1a} in rats using dose levels of 0, 0.25, 0.5 and 1.0 mg/kg bw per day, the NOAEL for maternal effects and embryo/fetal toxicity, including teratogenicity, was 1.0 mg/kg bw per day, the highest dose tested (Gordon, 1988a).

The Meeting concluded that the 8,9-*Z* isomer of avermectin B_{1a} is of no greater toxicity than the parent abamectin.

A metabolite, 24-hydroxymethyl abamectin, was found in liver and milk in smaller proportions than the parent. The Meeting concluded that 24-hydroxymethyl abamectin is of no greater toxicity than the parent, because it has been found in amounts of more than 10% in excreta and therefore has been tested in studies with the parent.

Human data

From reports on health records of manufacturing plant personnel, no adverse health effects were noted. A number of reports on intentional poisoning in humans available in the literature showed low susceptibility of humans to abamectin, with variable dose-related neurological signs and symptoms.

Although administration of abamectin to mice, rats and dogs at relatively low dose levels was associated with clinical signs of central nervous system toxicity (including mydriasis, tremors, convulsions, ataxia and bradycardia), it was shown that rhesus monkeys and humans are less sensitive to abamectin, at least following acute exposure (Gordon, 1985c).

The Meeting concluded that the existing database on abamectin was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting established a new ADI of 0–0.001 mg/kg bw, based on the NOAEL of 0.12 mg/kg bw per day for lower body weights and delayed time of vaginal opening observed at 0.20 mg/kg bw per day in post-weaning pups in the two developmental neurotoxicity studies in rats, using a safety factor of 100. The Meeting withdrew the existing ADI of 0–0.002 and also concluded that the additional information available to this Meeting regarding the critical period in development and species differences does not justify the application of a reduced safety factor to the NOAEL.

The ADI also applies to the 8,9-*Z* isomer of avermectin B_{1a} and the 24-hydroxymethyl metabolite of abamectin.

The Meeting established an ARfD of 0.003 mg/kg bw, based on the overall NOAEL of 0.25 mg/kg bw per day for clinical signs in dogs (mydriasis) observed in the first week of treatment at 0.5 mg/kg bw per day. This ARfD also applies to the 8,9-*Z* isomer of avermectin B_{1a} and the 24-hydroxymethyl metabolite of abamectin.

Levels relevant to risk assessment of abamectin and the 8,9-Z isomer of avermectin B_{1a}

Species	Study	Effect	NOAEL	LOAEL
Abamectin				
Mouse	Ninety-three-week study of toxicity and carcinogenicity[a]	Toxicity	4.0 mg/kg bw per day	8.0 mg/kg bw per day
		Carcinogenicity	8.0 mg/kg bw per day[b]	–
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	1.5 mg/kg bw per day	2.0 mg/kg bw per day
		Carcinogenicity	2.0 mg/kg bw per day[b]	–
	Two-generation reproductive toxicity study[c]	Reproductive toxicity	0.40 mg/kg bw per day[b]	–
		Parental toxicity	0.40 mg/kg bw per day[b]	–
		Offspring toxicity	0.12 mg/kg bw per day	0.40 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	1.6 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1.6 mg/kg bw per day[b]	–
	Acute neurotoxicity study[a]	Neurotoxicity	0.5 mg/kg bw	1.5 mg/kg bw
	Developmental neurotoxicity studies[a,d]	Developmental neurotoxicity	0.40 mg/kg bw[b]	–
		Toxicity	0.12 mg/kg bw	0.20 mg/kg bw
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day
		Embryo and fetal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day
Dog	Twelve-week,[a] 18-week[c] and 53-week[a] studies of toxicity[d]	Toxicity	0.25 mg/kg bw per day	0.5 mg/kg bw per day
8,9-*Z* isomer of avermectin B_{1a}				
CD-1 mice	Developmental toxicity study[c]	Maternal toxicity	3.0 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	3.0 mg/kg bw per day[b]	–
Rat	One-generation study of reproductive toxicity[a]	Reproductive toxicity	0.40 mg/kg bw per day[b]	–
		Parental toxicity	0.40 mg/kg bw per day[b]	–
		Offspring toxicity	0.40 mg/kg bw per day[b]	–

Species	Study	Effect	NOAEL	LOAEL
Rat	Developmental toxicity study[c]	Maternal toxicity	1.0 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1.0 mg/kg bw per day[b]	–

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.

Estimate of acceptable daily intake (ADI)

0–0.001 mg/kg bw

Estimate of acute reference dose (ARfD)

0.003 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to abamectin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, absorption almost complete, T_{max} 4–8 h
Dermal absorption	No data
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	> 80% within 96 h, almost exclusively in faeces
Metabolism in animals	Moderate to extensive; by demethylation, hydroxylation, cleavage of the oleandrosyl ring and oxidation reactions
Toxicologically significant compounds in animals and plants	Abamectin, 8,9-*Z* isomer of avermectin B_{1a}, 24-hydroxymethyl abamectin
Acute toxicity	
Rat, LD_{50}, oral	8.7 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 0.034 and < 0.21 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson and Kligman test)
Short-term studies of toxicity	
Target/critical effect	Nervous system/clinical signs, mortality
Lowest relevant oral NOAEL	0.25 mg/kg bw (dog)
Lowest relevant dermal NOAEL	No data

Lowest relevant inhalation NOAEC	0.5 µg/L (30-day rat study)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased mortality in males and reduced weight gain (mouse), clinical signs (rat)
Lowest relevant NOAEL	1.5 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)
Lowest relevant offspring NOAEL	0.12 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)
Developmental toxicity	
Target/critical effect	External malformations at maternally toxic dose (rabbit)
Lowest relevant maternal NOAEL	1.0 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	1.0 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	0.5 mg/kg bw (rat)
Subchronic neurotoxicity NOAEL	1.6 mg/kg bw per day (rat)
Developmental neurotoxicity NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)
Studies on 8,9-*Z* isomer of avermectin B_{1a} (photodegraded product)	
Developmental toxicity	
Target/critical effect	No developmental effects
Lowest relevant maternal NOAEL	3.0 mg/kg bw per day (highest dose tested; CD-1 mouse)
Lowest relevant embryo/fetal NOAEL	3.0 mg/kg bw per day (highest dose tested; CD-1 mouse)
Lowest relevant maternal NOAEL	1.0 mg/kg bw per day (highest dose tested; rat)
Lowest relevant embryo/fetal NOAEL	1.0 mg/kg bw per day (highest dose tested; rat)
Reproductive toxicity	
Target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)
Lowest relevant offspring NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)
Lowest relevant reproductive NOAEL	0.40 mg/kg bw per day (highest dose tested; rat)

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.001 mg/kg bw	Developmental neurotoxicity studies (rat)	100
ARfD	0.003 mg/kg bw	Twelve-, 18- and 53-week toxicity studies (dog)	100

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