

COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

IVERMECTIN (Modification of Maximum Residue Limits)

SUMMARY REPORT (5)

- Ivermectin belongs to the macrocyclic lactone class of endectocides and consists of a mixture of two homologous compounds, 22,23-dihydroavermectin B1a (H₂B1a, not less than 80%) and 22,23-dihydroavermectin B1b (H₂B1b, not more than 20%). Avermectins are potent anthelmintic, insecticidal and acaracidal compounds which, by increasing the membrane permeability to chloride ions, mediate the paralysis of the nematodes and certain classes of ectoparasites.

Initially, the Committee established an ADI of 0.2 µg/kg bw/day, i.e. 12 µg/person/day based on a NOEL of 0.1 mg/kg for maternotoxicity effects in a mouse teratogenicity study applying a safety factor of 500. In 1993, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated the ADI for ivermectin and concluded, on the basis of new human data, that the safety factor applied to this same NOEL could be reduced to 100, resulting in an ADI of 1 µg/kg bw/day corresponding to 60 µg/person/day. After reviewing this re-evaluation the CVMP established the same revised ADI.

Ivermectin is currently included in Annex I of Council Regulation (EEC) No 2377/90 with MRLs for bovine, porcine, ovine, *Equidae* and deer species as follows:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Ivermectin	22,23-Dihydro-avermectin B1a	Bovine	100 µg/kg 40 µg/kg	Liver Fat	
		Porcine, ovine, <i>Equidae</i>	15 µg/kg 20 µg/kg	Liver Fat	
		Deer, including reindeer	20 µg/kg 100 µg/kg 50 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	

- An application has now been submitted for the modification of the MRLs for bovine, porcine, ovine, *Equidae* and deer and requesting revision of the toxicological ADI to take into account data from human clinical trials in healthy volunteers and parasitised patients, and reports of individuals exposed to ivermectin as a result of accidental or deliberate ingestion as the basis for modification of the ADI. Summaries of safety data of ivermectin previously submitted and evaluated by the CVMP were also provided.

Ivermectin is a member of the macrocyclic lactone class, these substances bind selectively and with high affinity to glutamate-gated chloride ion channels which occur in invertebrate nerve and muscle cells. This leads to an increase in the permeability of the cell membrane to chloride ions with hyperpolarisation of the nerve or muscle cell, resulting in paralysis and death of the parasite. Compounds of this class may also interact with other ligand-gated chloride channels, such as those gated by the neurotransmitter gamma-aminobutyric acid (GABA).

Mammals do not have glutamate-gated chloride channels, the macrocyclic lactones have a low affinity for other mammalian ligand-gated channels and do not readily cross the blood-brain barrier.

3. Pharmacokinetic data were available from studies in mice, rats, dogs, primates and humans. In mice, peak plasma levels were reached approximately four hours after a single oral dose of 51 mg/kg bw. The average plasma to brain ivermectin concentration ratio was about 11 :1. When ivermectin was administered at 0.1 to 0.5 mg/kg bw for 35 days, steady-state concentrations were observed from day 21. The concentration in plasma and brain was proportional to the dose.

In rats administered oral doses of 0.06 to 0.75 mg H₂B_{1a} avermectin/kg bw, the dose and concentrations in plasma and tissues was well correlated. When oral doses of 0.3 mg [³H]ivermectin/kg bw were administered, tissue concentrations were highest in fat, followed by liver, kidney and muscle. The main route of excretion was via the faeces. Female rats received daily oral doses of 2.5 mg/kg bw for 61 days prior to mating until day 9 post-partum. Steady-state plasma concentrations were reached on day 10 of treatment. However, on day one post-partum, the plasma concentration was three to four times the steady-state concentration, probably due to increased mobilisation of fat. When treatment was restricted to days one to nine post-partum, plasma concentrations increased gradually throughout the lactation period, and concentrations in milk were at least three to four times the corresponding plasma concentrations. Neonatal plasma concentrations increased dramatically between days one to six post-partum, and on day 10 were up to three times higher than maternal plasma concentration. On days one and four post-partum, levels in neonatal brain tissue were similar to plasma concentrations. This suggested that the transfer of ivermectin via milk was probably responsible for the increase in neonatal mortality observed in multigeneration reproduction studies.

In a 36-day study in beagle dogs in which ivermectin was administered orally at 0.5 and 2.0 mg/kg bw, the concentrations of H₂B_{1a} in plasma increased dramatically between days two and eight and reached steady-state after about three weeks. A four-fold increase in the dose resulted in an average eight-fold increase in plasma levels.

In a comparative study with abamectin and ivermectin in immature rhesus monkeys, higher plasma concentrations were reached with ivermectin at all dose levels tested (2, 8 and 24 mg/kg bw). For both substances plasma concentrations were related to dose but the relationship was not linear. The highest plasma concentrations measured for the three doses of ivermectin were 110, 270 and 680 µg/l, respectively, whilst for abamectin, they were 76, 150 and 390 µg/l.

In a human volunteer study using various oral formulations, peak plasma concentrations were reached within approximately four hours. Administration of [³H]ivermectin showed that approximately 49 % of the dose was eliminated in faeces within five days. In a clinical study in lactating women treated with a single dose of ivermectin, a maximum concentration in milk of 23 µg/l was found the day after treatment. This level decreased to less than 0.1 µg/l about one week after treatment. Although plasma levels were not reported in this study and data determined from other studies were not directly comparable, it appears that concentrations in human milk are similar to or slightly less than those in plasma. Data from a modern clinical trial confirm the linearity of ivermectin pharmacokinetics in humans, they also indicate that bioavailability of ivermectin increases about 2.5 times when administered with a high fat meal, but is accompanied by a reduction in plasma half-life. In humans, the elimination t_{1/2} of 18 hours would result in a time to steady state of approximately 4 days of daily treatment. Using the dosing scheme as applied in the human volunteer study (administration on day 1, 3, 7), the time to reach steady state will be much longer than the maximum period used of 7 days. The plasma steady state concentration in humans would be at least 1.5-fold higher compared to the plasma levels achieved after administration on day 1, 3, and 7. The relation of these kinetics to central nervous system concentrations and differences in effect-sizes is unknown. Most studies on biotransformation were conducted with [³H]ivermectin. In *in vitro* studies with rat liver microsomes incubated with individual ivermectin components and an NADPH-regenerating system, more than 70% of the radioactivity was associated with the corresponding parent compound. The major polar metabolite was identified as the 24-desmethyl-hydroxymethyl alcohol.

These results corresponded well with those from *in vivo* liver metabolism studies. In addition, a group of non-polar metabolites was detected in fat, which yielded polar products on hydrolysis that were similar to the ivermectin metabolites present in liver.

4. Acute studies were conducted in mice, rats, rabbits, dogs and monkeys. The typical signs of acute ivermectin toxicity were all attributed to effects on the central nervous system. These were most severe in CF-1 mice, which exhibited ataxia, bradypnoea and tremors. Death occurred from approximately one to six hours after treatment. Ivermectin was more toxic in neonatal rats than in young adults. This was believed to be due to the postnatal completion of the blood-brain barrier in this species. In beagle dogs, mydriasis was the most sensitive indicator of toxicity. More severe signs included ataxia and tremors. Deaths were preceded by a comatose state. Approximately 30% of collie dogs were highly sensitive to ivermectin (as estimated from reports from non-recommended use of the drug). In immature rhesus monkeys no tremors or convulsions occurred. The most sensitive indicator was vomiting, which occurred in one of four animals given 2.0 mg/kg bw. The steep dose response curve in rodents was not reproduced in monkeys.

5. Short-term studies were conducted in rats, dogs and monkeys. In a 14-week study in rats ivermectin was administered orally to pregnant dams, splenic enlargement and bone-marrow hyperplasia was noted in offspring at 0.8 and 1.6 mg/kg bw. The NOEL was 0.4 mg/kg bw. These effects did not occur in other species.

In a 14-week oral study in beagle dogs (4/sex/group), mydriasis and slight weight loss were observed at 1.0 and 2.0 mg/kg bw. Four dogs in the 2.0 mg/kg bw group developed tremors, ataxia, anorexia and dehydration and were killed prior to the end of the study. The NOEL was 0.5 mg/kg bw.

In a two-week oral study, ivermectin was administered to neonatal monkeys at 0.04 and 0.1 mg/kg bw and to immature monkeys at 0.3, 0.6 and 1.2 mg/kg bw. No treatment-related effects were observed. In a short-term escalating dose study in monkeys, an NOEL of 1 mg/kg was identified.

6. Ivermectin is generally well-tolerated in the intended target species, with occasional coughing in sheep and goats after oral administration, occasional oedema and pruritis in horses and transient injection site pain in sheep. Neurotoxic effects similar to those seen in laboratory species may occur in overdose.
7. Three multigeneration studies were conducted in rats, but the first two were terminated because of neonatal toxicity at all doses. In the third (three-generation study), neonatal toxicity was observed at 0.4 mg/kg bw with increased neonatal mortality up to about 10 days post-partum and decreased bodyweight in the survivors. A cross-fostering study indicated that the neonatal toxicity was not related to *in utero* exposure but post natal exposure via maternal milk. There is evidence that neonatal rats are hypersusceptible to avermectin toxicity
8. The developmental toxicity of ivermectin was investigated in mice, rats, rabbits and dogs. The results demonstrated that teratogenic effects (cleft palates in mice, rats and rabbits, and clubbed fore-feet without skeletal alterations in rabbits) were produced only at doses similar to those causing severe maternal toxicity. The NOEL for teratogenicity in the most sensitive species and strain, the CF-1 mouse was 0.2 mg/kg bw, while the NOEL for maternal toxicity was 0.1 mg/kg bw. The CF-1 mouse has a genetic predisposition to avermectin toxicity. No teratogenic or maternotoxic effects were observed in dogs given oral doses of 0.5 mg/kg bw every five or ten days from days 5 to 40 of gestation.
9. Ivermectin was negative for mutagenic effects in a bacterial gene mutation study (up to 2000 µg/plate), an L5178Y mouse lymphoma assay (up to 1000 µg/ml), and a *Unscheduled DNA (deoxyribonucleic acid) Synthesis (UDS)* study in human IMR-90 fibroblasts (to 1000 µg/ml). The two components were negative in a bacterial gene mutation study.
10. No carcinogenicity studies were performed. However, such data were not considered necessary on the basis of the absence of structural alerts and the results of the mutagenicity studies.

11. No specific studies were provided concerning potential immunotoxicity. The results of laboratory animals studies and clinical use in humans gave no indications of any effect on the immune system.
12. No data on the effects of ivermectin of the human gut flora or micro-organisms used in food processing were available. However, such data were not considered necessary for this class of compound.
13. Ivermectin is widely used in humans for treatment of onchocerciasis and other parasitic diseases at single or repeated doses of 0.15 to 800 mg/kg bw. Tolerance to the compound has been assessed in healthy volunteers and in patients; adverse effects are usually mild and transient. In particular, no effects on the central nervous system were observed. The main effects noted in field and community based trials have been those arising from the death of the parasites, the so-called Mazzotti reaction, which is characterised by arthralgia, pruritis, fever, hypertension, tachycardia, headache and ocular changes. Neither in these studies nor during treatment for other parasitic diseases has a subset of atypically sensitive individuals been detected. Furthermore, the adverse effects experienced by the small number of persons accidentally exposed to doses (often of veterinary preparations) higher than customary human doses are in keeping with those noted in test animals.

A double blind, randomised, placebo controlled clinical trial was conducted to assess the safety and tolerability of oral subacute repeat doses of up to 1.2 mg/kg bw and an acute dose of 2.0 mg/kg bw in 68 healthy adult male and female human subjects as a treatment for headlice. No treatment related signs of toxicity were observed and in particular, there were no signs of neurotoxicity such as emesis, mydriasis (measured by pupillometry) or ataxia, that were predicted to be the most sensitive potential adverse effects based on the laboratory animal studies. A conservative NOEL of 420 µg/kg bw was identified.

However, it was considered that the human study could not be used to establish the ADI because the dosing regimen used. As indicated in paragraph 3 above, the time to reach steady state would be much longer than the maximum treatment period used (7 days). The plasma steady state concentration would be at least 1.5-fold higher than the levels achieved after administration on days 1, 3, and 7 only. The relation of these kinetics to central nervous system concentrations is unknown.

14. As indicated in the CVMP Summary Reports addressing the revision of the ADIs for abamectin (EMA/MRL/838/02-FINAL) and eprinomectin (EMA/MRL/520/98-FINAL), the CF-1 mouse is not directly relevant for human risk assessment due to the genetic predisposition of this strain to avermectin toxicity. The data from reproduction studies in the rat is also considered an unsuitable basis for setting an ADI, as there is evidence that neonatal rats are hypersusceptible to avermectin toxicity.

Therefore, because the rodent data are now considered inappropriate for ivermectin risk assessment, and the available human data were unsuitable, the dog toxicity data was considered the most relevant for the establishment of the toxicological ADI. In considering the pharmacokinetic data across species, the dog is seen as conservative – more sensitive than humans and non-human primates – in predicting ivermectin toxicity. A possible explanation for the greater sensitivity of the dog to ivermectin toxicity than humans is that there are clear differences in the oral pharmacokinetic profiles. In contrast to humans, a four-fold increase in dose from 0.5 to 2.0 mg/kg bw, results in over an 8.5-fold increase in plasma ivermectin concentrations. In healthy humans, single dose pharmacokinetic parameters show near linear behaviour at doses of 0.1 to 2.0 mg/kg bw. Ivermectin elimination in dogs is much slower than humans with a $t_{1/2}$ of 1.6 to 1.8 days, compared to 11.8 to 20.1 hours in humans. These data suggest that ivermectin may reach threshold levels for overt toxicity more readily in dogs than humans based on comparison of pharmacokinetics. Because of this it is considered that a safety factor of 50 is adequate for the establishment of an ADI based on the NOEL of 0.5 mg/kg bw/day in the 14-week dog study. This ADI is 10 µg/kg bw/day (600 µg/person per day).

The NOEL of 0.5 mg/kg bw/day from a 14-week repeat-dose study in dogs supports a new higher ADI in combination with an ivermectin-specific uncertainty factor that appropriately accounts for the uncertainties and species-specific differences associated with the use of the dog NOEL. The available human and non-human primate toxicological data supported the established uncertainty factor of 50.

The availability of new human safety data that accounts for the same central nervous system neuropharmacological interactions that are present in the dog, provides reassurance that the ADI established from the dog study is appropriate.

No new residue studies were submitted, a summary of those already available and previously evaluated in the different animal species are enclosed:

15. Four residue studies in cattle were presented. In three studies cattle were dosed percutaneously with 0.5 or 1.0 mg ivermectin/kg bw and in one study cattle were dosed subcutaneously with 0.2 mg/kg bw. These studies show that the highest concentrations of residues in tissues of cattle were found in liver followed by fat, kidney and muscle except for the injection site muscle. Joint FAO/WHO Expert Committee on Food Additives has calculated the percentage of the marker residue (22,23-dihydroavermectin B_{1a}) of the total residues in cattle 28 days post treatment. The marker accounted for 67% in muscle, 37% in liver, 54% in kidney and 18% in fat of total residues and the distribution of residues between tissues was 1:2:11:27 for muscle, kidney, fat and liver, respectively.
16. In a radiometric residue depletion study, 12 pigs were given a single tritium labelled (C_{22, 23} positions) ivermectin dose of 0.4 mg/kg bw subcutaneously. 3 animals in each group were killed on days 1, 7, 14 and 28 days after treatment. Fat total residue levels were the highest followed by liver. Total residue levels in fat were 384, 152, 28 and 6; in liver were 199, 112, 22, and 3 µg/kg on 1, 7, 14 and 28 days after treatment respectively. Kidney total residue levels were 106, 55 and 10 whereas muscle total residue levels were 43, 25 and 4 µg/kg after 1, 7 and 14 days respectively. The average marker to total residue ratios were 0.27, 0.41, 0.3 and 0.39 for liver, kidney, fat and muscle respectively.

In another radiometric residue depletion study, 15 pigs (male and female) were given tritium labelled (C_{22, 23} positions) ivermectin at a dose rate of 0.1 mg/kg bw/day for 7 days in the feed. Total radioactive residue levels in liver were 237.1, 43, 10.7, 4.1 and 2.7 µg/kg and those in fat were 207.2, 63.6, 18, 8 and 4.5 µg/kg at 4 hours, 3, 7, 14 and 21 days after treatment respectively.

In a residue depletion study where pigs were administered a single subcutaneous dose of 0.4 mg/kg bw, the highest residue levels were found at the injection site samples, followed by fat, liver, kidneys and then muscle at all time points. Mean injection site residues were 12500, 5100, 1110, 2300, 2500 and 230 µg/kg at 1, 3, 5, 7, 10 and 14 days after treatment respectively. Peak residue levels were found after 3 days in all of the other tissues. Liver residue levels were 67, 69, 53, 41, 23 and 13 µg/kg and fat residue levels were 74, 110, 91, 73, 47 and 24 µg/kg on days 1, 3, 5, 7, 10 and 14 days after treatment respectively.

17. In a radiometric residue depletion study, 12 sheep were given a single oral (intraruminal) tritium labelled (C_{22, 23} positions) ivermectin dose of 0.3 mg/kg bw. Three animals were killed on days 1, 3, 5 and 7 days after treatment. The average total concentration in liver were 238, 125, 25, 44 µg/kg at 1, 3, 5 and 7 days after treatment respectively. At the same time points, average total residue concentrations in fat were 307, 153, 63, 73; in kidney 72, 46, 12, 13 and in muscle were 55, 50, 9 and 10 µg/kg. The ratios of marker (H₂B_{1a}) to total residues at 3 days post treatment were 0.51, 0.51, 0.44 and 0.52 for liver, fat, kidney and muscle, respectively.

In a residue depletion study, 30 sheep (male and female) were given oral doses of 0.3 mg ivermectin/kg bw in a micellar vehicle. The highest residues (marker residue, H₂B_{1a}) were found in fat followed by liver. Residue levels in liver were 72, 12, 11 and 8 µg/kg; in fat were 145, 32, 11 and 9 µg/kg; in kidneys were 30, 5, 2 and 1 µg/kg and in muscle were 20, 4, 2 and 2 µg/kg at 1, 3, 5 and 7 days after treatment respectively.

In a residue depletion study where sheep were administered a three subcutaneous doses of 0.3 mg/kg bw at weekly intervals, the highest residue levels were found at the injection site samples, followed by fat, liver, kidneys and then muscle at all time points. Mean injection site residues were 17000, 2900, 2300, 460 and 220 µg/kg at 3, 7, 10, 14 and 28 days after last treatment respectively. Peak residue levels were found after 7 days in all of the other tissues. Liver residue levels were 160, 190, 97, 55 and 7.2 µg/kg and fat residue levels were 230, 310, 180, 99 and 13 µg/kg at 3, 7, 10, 14 and 28 days after treatment respectively.

18. In a radiometric residue depletion study, 3 horses were given tritium labelled (C_{22, 23} positions) ivermectin doses of 0.3 mg/kg bw. Two of the animals were administered orally and the remaining one was administered intramuscularly. The animals were then slaughtered after 28 days and the mean total radioactivity levels in those administered orally was 2.64, 3.02, 3.1, 4.26, 4.11 and 3.52 µg/kg and in those administered intramuscularly was 43.2, 17.1, 14.4, 54.2, 47.4 and 36.1 for liver, kidneys, muscle, perirenal fat, omental fat and subcutaneous fat respectively. Total radioactivity levels at the injection site was 64.4 µg/kg. The ratios of marker to total residues were 0.12, 0.22 and 0.36 for liver, kidney and fat, respectively.

In a residue depletion study where horses were administered a single oral dose of 0.3 mg/kg bw, the highest residue levels were found fat followed by liver. Fat residues were 80 and 10.9 µg/kg and liver residues were 31 and 4 µg/kg on 7 and 14 days after treatment. Residue levels were only detected after 7 days in kidney (15 µg/kg) and muscle (8.3 µg/kg).

19. Following a single percutaneously administered dose of 1 mg/kg (twice the recommended dose) to red deer, the tissue residues decreased progressively with time with highest concentrations found in fat followed by liver, muscle and kidney. The ratio of 22,23-dihydroivermectin B1a in tissues at 28 days withdrawal, were in this study 6.8:2.5:1.3:1 for fat, liver, kidney and muscle. At 7 and 28 days after treatment the mean concentrations of 22,23-dihydroivermectin B1a of residues in fat were 292 µg/kg and 13.2 µg/kg, respectively, in liver 180 µg/kg and 9.3 µg/kg, respectively, in muscle 78 µg/kg and 1.4 µg/kg respectively and in kidney 78 µg/kg and 3.6 µg/kg, respectively.

A single subcutaneous dose of 0.2 mg ivermectin/kg bw was given to reindeer. The ratio of 22,23-dihydroivermectin B1a in tissues at 17 days withdrawal, were in this study 9.5:6.6:2.6:1 for fat, liver, kidney and muscle, respectively. The highest residue concentrations of 22,23-dihydroivermectin B1a 10 and 17 days after treatment, respectively, were 362 µg/kg and 68 µg/kg in back fat, 71 µg/kg and 28 µg/kg in liver, 54 µg/kg and 13 µg/kg in kidney, 40 µg/kg and 11 µg/kg in muscle, and 44 µg/kg and 9 µg/kg in injection site muscle. The half-lives in the tissues after a single subcutaneous treatment were 7.1 days in back fat, 2.9 days for the injection site, 4.9 days for muscle, 5.8 days for liver and 5.7 days for kidney.

20. A routine analytical method validated in accordance with Volume 8 of the Rules Governing Medicinal Products in the European Union based on HPLC with fluorescence detection was presented in an internationally recognised format for quantifying 22,23-dihydro-ivermectin B1a residues in tissues from bovine, porcine, ovine, *Equidae* and deer. The limit of quantification for all tissues for pigs, *Equidae* and ovine species was 5 µg/kg. For bovine species the limit of quantification was 3 µg/kg for muscle, 5 µg/kg for fat and kidney and 3.6 µg/kg for liver. For deer the limit of quantification was 2 µg/kg for all tissues. This method should be applicable to other mammals.
21. In accordance with the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL) it was possible to extrapolate the MRLs to all mammalian food producing species.

Conclusions and recommendation

Having considered that:

- a revised toxicological ADI of 10 µg/kg bw/day (600 µg/person per day) was established,
- residues in edible tissues in cattle, pigs, sheep, horses and deer are below the ADI from early time points,
- 22,23-dihydroavermectin B_{1a} (H₂B_{1a}) is the marker residue in all tissues and species,
- the tissue distribution of residues and the overall ratios of marker to total residues were generally similar with residue levels being the highest in fat and liver tissues. Horses and pigs had slightly different marker/total residue ratios,
- residue concentrations were persistently low in non injection site muscle, therefore muscle was considered unsuitable for monitoring purposes and no MRLs were established for muscle,
- an analytical method for the monitoring of residues in tissues of all mammalian food producing species was available;

the Committee recommends the modification of the current entries for ivermectin for bovine, porcine, ovine, *Equidae* and deer, including reindeer, in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissue	Other provisions
Ivermectin	22,23-Dihydro-avermectin B _{1a}	All mammalian food producing species	100 µg/kg 100 µg/kg 30 µg/kg	Fat Liver Kidney	Not for use in animals producing milk for human consumption.

Based on these MRLs, the daily intake of total residues will represent about 15% of the ADI.