



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

EMAMECTIN

SUMMARY REPORT (1)

1. Emamectin is an avermectin that is structurally very similar to eprinomectin, the only difference being the presence of an epi-methylamino group at the C4'' position in the emamectin molecule rather than an epi-acetylamino group at that position in the case of eprinomectin. Emamectin consists of 90% or more of the B1a component (ethyl group on C₂₆ of the molecule) and not more than 10% of the B1b component (methyl group on C₂₆). The benzoate salt is being developed for the treatment of sealice infestations in *Salmonidae*. The substance would be administered orally, in the feed, at a dose equivalent to 50 µg/kg bw per day (expressed as benzoate salt) for 7 days.
2. The precise mode of action of emamectin remains unknown despite extensive investigations with other substances in the same class.
3. A series of experiments was carried out to determine the fate of the B1a component of emamectin after administration of the benzoate salt to Sprague-Dawley rats. In the first experiment, rats were given a single oral dose of 20 mg/kg bw dual-labelled [¹⁴C]/[³H]-emamectin B1a and killed 7 days later; the residues in tissues were very similar when based upon ¹⁴C and ³H radioactivity indicating stability of the ³H-label. Following administration of a single oral dose of 0.5 mg/kg bw [¹⁴C]-emamectin B1a, mean peak plasma concentrations of approximately 17 and 21 µg equivalents/kg bw were attained 12 hours and 4 hours after administration to males and females respectively. The oral bioavailability was estimated to be approximately 55% in males and 74% in females and the half-life of plasma elimination was approximately 34 hours in males and 51 hours in females. When the same dose was administered intravenously, the half-life of plasma elimination was 29 hours in males and 41 hours in females. The substance was widely distributed to the tissues. Seven days after oral administration of 20 mg/kg bw [¹⁴C]/[³H]-emamectin B1a, tissue residues ranged from 8 to 2033 µg-equivalents/kg in the following order: most being present in lung followed by gastro-intestinal tract, kidney, liver, fat, bone, muscle, spinal cord, blood and least residues in the brain. Tissue residues were much lower in rats given 0.5 mg/kg bw. In both sexes, regardless of route of administration, more than 94% of the administered dose was eliminated in faeces and less than 1% in the urine.
4. In rats, approximately 80% of the radiolabelled material in faeces and in tissues was unmetabolised emamectin B1a. An N-demethylated product of emamectin B1a, 4''-deoxy-4''epiaminoavermectin B1a, was the only metabolite found in faeces, liver, kidney, muscle and fat. The amount of this metabolite in faeces increased with time post-dosing. In faeces, this metabolite represented approximately 1 to 2% of the radioactivity on day one after dosing but 18 to 19% on day 7 after dosing. The percentage of this metabolite found in faeces was independent of the dose level administered, the route of administration or the sex of the animal.

5. Some of the toxicity studies were carried out using the hydrochloride salt of emamectin. Other studies were carried out using 2 different forms of the benzoate salt: the hydrate and the methyl t-butyletherate solvate (also referred to as the solvate). The relative bioavailabilities of these different salts were not given. In all the safety studies, the doses were quoted as mg/kg bw emamectin base
6. Acute oral LD₅₀ values for emamectin benzoate salt solvate and emamectin benzoate hydrate salt were 120 mg/kg bw and 107 mg/kg bw respectively in female CD-1 mice. The acute oral LD₅₀ for both benzoate salts was 89 mg/kg bw in female Sprague-Dawley rats. The hydrochloride salt was of similar toxicity in Sprague-Dawley rats with acute oral LD₅₀ values of 88 and 76 mg/kg bw in males and females, respectively. However in CF-1 mice, the acute oral LD₅₀ of the hydrochloride salt was 22 mg/kg bw and 31 mg/kg in males and females respectively. Similar signs of toxicity were observed in all the acute toxicity studies and included tremors, ataxia, decreased activity, ptosis, bradypnoea and loss of righting reflex. Additionally, in rats, irritability, salivation and lacrimation were also observed.
7. Further acute toxicity studies were carried out to investigate neurotoxic potential. In these studies, the brains, spinal cords, sciatic and optic nerves were examined microscopically at termination. Sprague-Dawley rats were given oral doses of the hydrochloride salt ranging from 27.4 to 82.2 mg/kg bw; clinical signs of toxicity together with degeneration of the brain, spinal cord and sciatic nerve were observed at all dose levels. Rats of the same strain were given oral doses of the benzoate salt ranging from 0.5 to 25 mg/kg bw. Tremors and irritability were observed at 10 mg/kg bw and an increased incidence of degeneration of the brain, spinal cord and sciatic nerve was observed at 25 mg/kg bw; the NOEL was 5.0 mg/kg bw. Following topical administration of 500, 1000 or 2000 mg/kg bw to New Zealand White rabbits, tremors were observed in one rabbit after a 4-hour exposure at the lowest dose level and tremors and mydriasis were observed in the majority of the rabbits at all dose levels following a 24-hour exposure. At termination, treatment-related changes were observed in all groups and included white matter degeneration in the optic chiasm, pons and/or cerebellar penduncles, white matter degeneration in the lateral and ventral funiculi of the spinal cord and degeneration in the peripheral nerve. In contrast, no treatment-related changes were observed following a 4-hour nose-only inhalational exposure of Sprague-Dawley rats to atmospheric concentrations of 0.01 to 0.10 mg/litre of air.
8. In a preliminary study in CD-1 mice, groups of 10 animals per sex and dose were fed diets calculated to provide intakes of 0, 0.2, 0.6, 1.2 or 2 mg/kg bw/day of emamectin (as hydrochloride salt) for 13 days. There were no signs of toxicity and no histopathological changes in the brain, spinal cord or sciatic nerve at any dose level. In another study, groups of 10 animals per sex and dose CF-1 mice were fed diets calculated to provide intakes of 0, 0.05, 0.1, 0.3 or 0.9 mg/kg bw/day of emamectin (as benzoate salt) for 15 days. Signs of toxicity (including tremors, decreased activity, slow righting reflex, reduced body weight and food consumption) were observed at 0.3 mg/kg bw and above and several mice from the 0.3 and 0.9 mg/kg bw groups were euthanased in a moribund condition. No haematology or blood chemistry investigations were carried out. Histopathological examinations were limited to the brain, spinal cord and sciatic nerve; there were no substance-related effects at any dose level. The NOEL was 0.1 mg/kg bw/day, based on neurotoxicity.
9. Groups of 20 Sprague-Dawley rats per sex per dose were fed diets calculated to provide intakes of 0, 0.5, 2.5 or 12.5 mg/kg bw/day of emamectin (as hydrochloride salt) for up to 14 weeks. The top dose of 12.5 mg/kg bw/day was reduced to 8 mg/kg bw/day during week 3, and subsequently to 5 mg/kg bw/day during week 9. During weeks 3 to 11, 9 out of 20 males given the top dose were euthanased due to toxicity. In this group, whole body tremors were observed in both sexes and continued when the dose was reduced. There was a significant reduction in body weight gain in the top dose group throughout the study. Treatment-related neuronal vacuolation was found in the brains from 2 out of 20 male rats given 2.5 mg/kg bw and the majority of rats given the top dose. Most rats in the top dose group also exhibited neuronal vacuolation/degeneration of the spinal cord and sciatic nerve together with skeletal muscle atrophy. The NOEL was 0.5 mg/kg bw/day.

10. A second 14-week study in Sprague-Dawley rats was designed to investigate neurotoxicity and incorporated a Functional Observational Battery and Motor Activity testing during weeks 5, 9 and 13/14. Groups of 10 rats per sex per dose were fed diets calculated to provide intakes equivalent to 0, 0.25, 1.0 or 5.0 mg/kg bw/day of emamectin (as benzoate salt hydrate). Tremors were observed at 5 mg/kg bw and body weight and food consumption were significantly reduced at this dose level. In the Functional Observational Battery, parameters such as posture, gait, rearing, grip strength and righting reflex were adversely affected in the 5 mg/kg bw group with males more severely affected than females. At termination, the rats were anaesthetised and their tissues perfused; tissue sampling was limited to the central and peripheral nervous systems, optic nerve and skeletal muscle. All males and most females in the 5 mg/kg bw group showed neuronal vacuolation/degeneration of the brain, spinal cord and sciatic nerve. The NOEL was 1.0 mg/kg bw/day.
11. Groups of 20 Sprague-Dawley rats per sex per dose were fed diets calculated to provide intakes equivalent to 0, 0.1, 1.0 or 2.5 mg/kg bw/day of emamectin (as benzoate salt hydrate) for 53 weeks. Females receiving the top dose were given 5 mg/kg bw/day up to week 18 when the dose was reduced to 2.5 mg/kg bw. Top dose males received 2.5 mg/kg bw throughout. A Functional Observational Battery and Motor Activity testing were carried out during weeks 13, 24, 38 and 51. Tremors and reduced body weight gain were observed in females given 5 mg/kg bw; both effects were ameliorated when the dose was reduced to 2.5 mg/kg bw. In the Functional Observational Battery treatment-related changes were observed in females given 5 mg/kg bw and males given 2.5 mg/kg bw. At termination, neuronal degeneration of the brain and spinal cord was observed in males given 2.5 mg/kg bw and the females given 5/2.5 mg/kg bw. The NOEL was 1.0 mg/kg bw/day.
12. In an exploratory study, groups of Beagle dogs were given daily oral doses of 0, 0.5 or 1.5 mg/kg bw per day of emamectin (as hydrochloride) for up to 5 weeks. At necropsy, the dogs were anaesthetised and perfused *via* the left ventricle. Sections of the brain, spinal cord and sciatic nerve were examined by electron microscopy. Sections were also stained using a monoclonal antibody system designed for the detection of phosphorylated epitopes on neurofilaments. This study showed that neuronal injury was associated with the accumulation of phosphorylated neurofilaments in the neuronal perikaryon and first became apparent after 2 weeks of treatment. This was followed by axonal degeneration and breakdown of the associated myelin; these changes were also apparent after 2 weeks but were more obvious after 4 weeks due to the additional accumulation of myelin. These changes were detected by both routine light microscopy and immunohistochemistry after 2 weeks in the 1.5 mg/kg bw group. The NOEL was 0.5 mg/kg bw/day.
13. Groups of 4 Beagle dogs per sex per dose were given daily oral gavage doses of 0, 0.5, 1 or 1.5 mg/kg bw/day of emamectin (as hydrochloride salt) for 14 weeks. The doses were reduced to 0.25, 0.5 and 1 mg/kg bw/day respectively by the start of week 3. A total of 3 dogs in the 1.5/1 mg/kg bw group were euthanased; prior to death, these dogs showed tremors, mydriasis, anorexia and lethargy. Body weight gain and food consumption were reduced in the 1.5 mg/kg bw/day group but improved when the dose was reduced to 1 mg/kg bw/day. At termination, neuronal degeneration was found in the brains of dogs receiving 1.5 mg/kg bw/day followed by the reduced dose of 1 mg/kg bw/day and 1 mg/kg bw/day followed by the reduced dose of 0.5 mg/kg bw/day. Scattered neuronal vacuolation was noted in the spinal cords from all animals given 1.5 mg/kg bw/day followed by the reduced dose of 1 mg/kg/day, and one male given 1 mg/kg bw/day followed by the reduced dose of 0.5 mg/kg bw/day. Sciatic and optic nerve lesions, which consisted of scattered vacuolation, were seen in the majority of animals receiving 1.5 mg/kg bw/day followed by the reduced dose of 1 mg/kg bw/day. Skeletal muscle atrophy was seen in 7 of 8 dogs given 1 mg/kg bw/day followed by the reduced dose of 1.5 mg/kg bw/day, and 2 of 8 dogs given 0.5 mg/kg bw/day followed by the reduced dose of 1 mg/kg bw/day. The NOEL was 0.25 mg/kg bw/day, based on neuronal degeneration, and skeletal muscle atrophy in dogs receiving higher doses.

14. Groups of 4 Beagle dogs per sex per dose were given daily oral gavage doses of 0, 0.25, 0.5, 0.75 or 1 mg/kg bw/day of emamectin (as benzoate salt solvate) for 53 weeks. Due to severe toxicity, in the form of body tremors, mydriasis, decreased motor activity, reduced food consumption and body weight, all dogs given 1 mg/kg bw/day were euthanased after receiving 19 doses and all males given 0.75 mg/kg bw/day were euthanased after receiving 49 doses. The females given 0.75 mg/kg bw/day showed similar signs of toxicity but of decreased severity. One out of 8 dogs in the group treated with 0.5 mg/kg bw/day also displayed tremors. In the central nervous system, neuronal degeneration was reported in males given 0.75 mg/kg bw/day, and both sexes given 1 mg/kg bw/day. Axonal degeneration in the central and peripheral nervous system was seen at doses of 0.5 mg/kg bw/day and above. Degeneration of the retinal ganglionic cells and axonal degeneration in the optic nerve were noted at doses of 0.75 and 1 mg/kg bw/day. The NOEL was 0.25 mg/kg/day. The same NOEL was established in dogs following dosing for 14 weeks and 53 weeks even though the salts of emamectin were different.
15. Atlantic salmon were kept in tanks supplied with seawater under continuous flow conditions. They were fed diets containing emamectin equivalent to actual doses of 0, 70, 173 or 356 µg/kg bw/day for 7 days. Signs of toxicity were observed only in the group receiving 356 mg/kg bw (corresponding to approximately 7 times the recommended dose) and included one death, lethargy, dark discolouration, inappetance and lack of co-ordination.
16. Groups of mated female Sprague-Dawley rats were given daily oral gavage doses of 0, 0.1, 0.6 or 3.6 mg/kg bw/day of emamectin (as benzoate salt hydrate), commencing on day 6 of gestation and continuing up to day 20 of lactation. Between days 17 and 20 of gestation, the top dose was reduced to 2.5 mg/kg bw/day, due to tremors observed in pups given this dose in a concurrent study. No adverse effects were observed on the dams. Tremors and hindlimb extension and reduced body weight gain were observed in the F1 pups in the group given 3.6 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day. Behavioural tests were carried out on the pups during lactation and during the post-weaning period. Effects were observed only in the group given 3.6 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day and included increased stereotypic behaviour and decreased auditory startle response. Some signs of toxicity including behavioural effects and reduced body weight in comparison with the controls were still observed in this group during the post-weaning period. Also in the top dose group, vaginal canalization and preputial separation were delayed by 3 to 4 days in comparison with the controls. The NOEL was 0.6 mg/kg bw/day.
17. In a two generation reproduction study, male and female Sprague-Dawley rats were fed diets calculated to provide intakes of 0, 0.1, 0.6 or 3.6 mg/kg bw/day of emamectin (as benzoate salt hydrate). Three weeks after weaning of the F1a pups, the F0 generation was mated for a second time; pairs of rats which did not produce a pregnancy following the first cohabitation were paired with fertile animals of the opposite sex. The 3.6 mg/kg bw/day dose was reduced to 1.8 mg/kg bw/day on gestation day 0, following the second cohabitation of the F0 females, in order to examine the effect of reducing the dosage on the severity of the effects on the pups. There were significant reductions in weight gain in the F0 males and females given 3.6 mg/kg bw/day and food intake was reduced during the lactation period in F0 females given 3.6 mg/kg bw/day. At necropsy, neuronal degeneration of the brain and/or spinal cord was found in most rats of both sexes from the group given 3.6 mg/kg bw/day followed by the reduced dose of 1.8 mg/kg bw/day. In the F1a litters, the percentage of live pups per litter was significantly reduced in the group given 3.6 mg/kg bw/day. During lactation, the F1a pups in the 3.6 mg/kg bw group showed tremors and hind limb extension or splay; these effects continued to be observed during the post-weaning phase. In the F1b litters, in which the top dose was reduced to 1.8 mg/kg bw, the severity of the clinical signs in the pups was reduced. In the group given 3.6 mg/kg bw/day followed by the reduced dose of 1.8 mg/kg bw/day, there were significant decreases in both the fecundity and fertility indices at the F2 mating. Signs of toxicity were observed in only one litter from the 1.8 mg/kg bw group from this mating. The NOEL was 0.6 mg/kg bw/day, based on reduced body weight gain, food intake, fecundity and fertility rate and neuronal degeneration at higher doses.

18. Groups of 25 pregnant Sprague-Dawley rats were given daily oral gavage doses of 0, 2, 4 or 8 mg/kg bw/day of emamectin (as benzoate salt solvate) from days 6 to 19 of gestation. Signs of maternal toxicity such as tremors and convulsions were observed in dams given 8 mg/kg bw/day. There was a significant dose-related reduction in maternal body weight gain in the 4 and 8 mg/kg bw groups during the latter parts of the study. There was no evidence of teratogenicity at any dose level. In the 8 mg/kg bw group, there was a significant increase in the number of fetuses with supernumerary ribs and in the incidence of delayed ossification. The NOEL for foetal toxicity was 4 mg/kg bw/day.
19. Groups of 18 inseminated pregnant female New Zealand White rabbits were given daily oral gavage doses of 0, 1.5, 3 or 6 mg/kg bw/day of emamectin (as benzoate salt solvate) from gestation days 6 to 18. (The dose levels were selected following a range-finding study in which the top dose level of 8 mg/kg bw/day induced malformations in 2 fetuses from different litters. The malformations were cleft palate and/or hydrocephalus and the fetuses were from dams which had suffered tremors and/or body weight loss.) Maternal body weight gain and food consumption were reduced in the 6 mg/kg bw group and these dams had mydriasis and decreased pupillary reactions. There was no evidence of teratogenicity or foetotoxicity at any dose level. The NOEL for maternal toxicity was 3 mg/kg bw/day.
20. Emamectin was not mutagenic in *in vitro* assays for gene mutation in *Salmonella typhimurium* TA97a, TA98, TA100 and TA1535, in *Escherichia coli* WP2, WP2 uvrA and WP2 uvrA pKM101 and in a gene mutation assay at the HPRT locus of V-79 Chinese hamster lung cells. Negative results were obtained in a chromosomal aberration assays in Chinese hamster ovary cells but an *in vitro* test for DNA-damage in rat hepatocytes measured by alkaline elution gave positive results. Negative results were obtained in an *in vivo* metaphase analysis in which mice were given single oral doses of 8, 26 or 80 mg/kg bw and the bone marrow harvested 6, 24 and 48 hours after dosing. It was concluded that emamectin was not genotoxic.
21. In a combined carcinogenicity and chronic toxicity study, groups of 75 Sprague-Dawley rats per sex per dose were fed diets calculated to provide intakes of 0.25, 1 or 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day of emamectin (as benzoate salt hydrate) for 105 weeks. Groups of 130/sex control rats were fed untreated diets. The dose of 5 mg/kg bw/day was reduced to 2.5 mg/kg bw/day in week 6 for males, and week 10 for females. Body weight gain and food consumption were significantly increased at 1 mg/kg bw/day and above during the first year of the study but a significant reduction was observed in the group given 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day during the second year of the study. In the groups given 1 and 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day, serum triglyceride levels were elevated in both sexes and serum bilirubin was elevated in females. There was no evidence of carcinogenicity. At necropsy, neuronal vacuolation was observed in the brain and spinal cord of both sexes given 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day. There was an increased incidence of diffuse vacuolation of hepatocytes in female rats given 1.0 and 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day and an increase in chronic proliferative cystitis in the urinary bladder of males given 5 mg/kg bw/day followed by the reduced dose of 2.5 mg/kg bw/day. The NOEL was 0.25 mg/kg bw/day, based on body weight changes and increased serum triglyceride and bilirubin concentrations. The NOEL based on neurotoxicity was 1 mg/kg bw/day.

22. In a carcinogenicity study, groups of 50 CD-1 mice per sex per dose were fed diets calculated to provide intakes of 0.5, 2.5 or 12.5 mg/kg bw/day of emamectin (as benzoate salt hydrate) for 79 weeks. Two control groups each of 50 per sex mice were fed untreated diets. The dose of 12.5 mg/kg bw/day was reduced to 7.5 mg/kg bw/day in females during week 48, and to 7.5 mg/kg bw/day in males during week 9, and further reduced to 5.0 mg/kg bw/day in males during week 31, due to weight loss at these dose levels in a concurrent study. Satellite groups of 15 rats per sex per dose rats were fed the same diets and used for haematological examinations. Overall mortality was significantly increased in both sexes given 12.5 mg/kg bw/day followed by the reduced doses of 7.5 and 5.0 mg/kg bw/day. Tremors and vocalisation was noted in some mice given 12.5 mg/kg bw/day followed by the reduced doses of 7.5 and 5.0 mg/kg bw/day from week 5 onwards and several mice in this group showed fine forelimb fasciculating tremors from week 14 onwards. Body weight gain was significantly reduced in the top dose group. There was no evidence of carcinogenicity. In 2 males given 12.5 mg/kg bw/day, sciatic nerve degeneration, characterised by vacuolation and presence of myelin balls in the nerve fibres was noted. The NOEL was 2.5 mg/kg bw/day, based on neurotoxicity and decreased weight gain in mice receiving higher doses.
23. Emamectin is being developed for use in veterinary medicine and no data concerning the potential effects in humans were available.
24. No data were provided concerning the potential effects of emamectin on the human gut flora or the microorganisms used in industrial food processing. It was agreed that such data were not needed for the avermectins.
25. An Acceptable Daily Intake of 1 µg/kg bw/day was established by applying a safety factor of 100 to the NOEL of 100 µg/kg bw/day which was established in the 15-day repeated-dose toxicity study in CF-1 mice, based on neurotoxicity.
26. Atlantic salmon postmolts were held in a tank supplied with running seawater at a temperature of approximately 7°C. Individual fish were caught and dosed orally with a syringe. ³H-emamectin was administered as a single dose equivalent to 42 µg equivalents/kg bw. The fish were killed (2 per time-point) and sections were cut, freeze-dried and prepared for autoradiography. From the remaining material, samples of various tissues were taken and the residues determined using scintillation counting. At 4 hours, autoradiography indicated some radioactive material in the stomach and intestinal lumen but little absorption. By 12 hours, significant amounts were present in the gut epithelium, the liver and the kidney. Liquid scintillation counting indicated peak concentrations of radioactivity 2 to 7 days after dosing. Highest concentrations were found in the bile (788 µg equivalents/kg at 42 days), liver (342 µg equivalents/kg at 7 days) and kidney (361 µg equivalents/kg at 21 days). Concentrations were lower in muscle (peak value 13 µg equivalents/kg at 4 days), skin (peak value 19 µg equivalents/kg found at both 2 days and 28 days) and brain (peak value 13 µg equivalents/kg at 7 days).
27. Atlantic salmon, 28-months of age, were held in tanks of re-circulating artificial seawater at a temperature of approximately 10°C. The fish were given treated feed at a nominal dose of 50 µg/kg bw/day emamectin benzoate for 7 days. The substance was administered as a mixture of unlabelled emamectin benzoate (95% B1a and 5% B1b) together with ³H-emamectin benzoate B1a. The fish were killed (3 per time point) at intervals from 3 hours up to 45 days after the final dose. Total residues were determined using liquid scintillation counting. Highest residues were found in kidney 12 hours after the last dose (mean residues 2920 µg equivalents/kg, declining to 890 µg equivalents/kg on day 45). Residues in muscle peaked at a mean value of 74 µg equivalents/kg 12 hours after the last dose and declined to 17 µg equivalents/kg at day 45. Residues in skin peaked at a mean value of 132 µg equivalents/kg at 12 hours after the last dose and declined to 28 µg equivalents/kg at day 45. Tissues were extracted with methanol and analysed using HPLC. The major component in all tissues was unmetabolised emamectin B1a. Residues of emamectin B1a in muscle were 67 µg/kg (mean value) 12 hours after the last dose (91% of total residues) and declined to 20 µg/kg at 30 days (82.5% of total residues). Residues of emamectin B1a in skin were 124 µg/kg (mean value) 12 hours after the last dose (93.7% of total residues) and declined to 39 µg/kg at 30 days (88.4% of total residues).

28. Atlantic salmon, 30 months of age, were held in tanks of re-circulating artificial seawater at a temperature of approximately 4.8°C. The fish were given treated feed at a nominal dose of 50 µg/kg bw per day emamectin benzoate for 7 days. The substance was administered as a mixture of unlabelled emamectin (95% B1a and 5% B1b) together with ³H-emamectin benzoate B1a. The fish were killed (10 per time point) at intervals from 3 hours up to 90 days after the final dose. Total residues were determined using liquid scintillation counting. The residues peaked at later time-points than in the previous study in which a higher water temperature was employed. Highest residues were found in kidney (mean residues 3065 µg equivalents/kg 15 days after the last dose declining to 1436 µg equivalents/kg on day 90) and liver (mean residues 2260 µg equivalents/kg 15 days after the last dose declining to 1083 µg equivalents/kg on day 90). Mean residues in muscle were in the range 53 to 65 µg equivalents/kg during the first 72 hours and declined to 19 µg equivalents/kg on day 90. Mean residues in skin were in the range 69 to 93 µg equivalents/kg during the first 72 hours and declined to 36 µg equivalents/kg on day 90. Mean residues in muscle + skin samples were in the range 55 to 64 µg equivalents/kg during the first 72 hours and declined to 20 µg equivalents/kg on day 90. Methanolic extracts of liver, kidney, muscle, skin and intact muscle with skin were prepared for radio-HPLC analysis. The major component in all tissues, accounting for at least 80% of the total residues, was unmetabolised emamectin B1a. In samples of muscle with skin, emamectin B1a accounted for 98% of the total residues at 12 hours; this proportion declined to 83% at day 90. Over this time period, the mean residues of emamectin B1a in muscle with skin declined from 76 µg/kg to 19 µg/kg. The N-demethylated metabolite of emamectin, 4''-deoxy-4''-epi-amino-avermectin B1, was undetectable in samples of muscle with skin at 12 hours but accounted for 6% of the total residues (3.5 µg/kg) at 7 days and 15% (3.6 µg/kg) at 90 days. The metabolite 4''-deoxy-4''-epi-(N-formyl-N-methyl)amino-avermectin B1 comprised approximately 1% of the total residues in muscle with skin at 12 hours and 7 days but was undetectable at later time points. The data indicated that emamectin B1a was a suitable marker residue.
29. The proposed routine analytical method for determination of residues of emamectin B1a in fish muscle and skin was based on HPLC with fluorescence detection. The method was described in the ISO 78/2 format. The specificity of the method was acceptable and residues of ivermectin did not interfere with the analysis. The limit of quantification for emamectin B1a was 61 µg/kg for salmon muscle and skin in natural proportions and the limit of detection was 2.5 µg/kg.

Conclusions and recommendation

Having considered that:

- an ADI of 1 µg/kg bw (i.e. 60 µg/person) was established for emamectin base,
- emamectin B1a was identified as the marker residue in fish muscle and skin and comprised approximately 90% of the total residues during the period 12 hours to 30 days after treatment, the amount depending on water temperature,
- a validated analytical method for the determination of residues of emamectin B1a in fish tissues was available;

the Committee for Veterinary Medicinal Products recommends the inclusion of emamectin in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Emamectin	Emamectin B1a	Salmonidae	100 µg/kg	Muscle and skin in natural proportions	

Based on these MRLs, the daily intake of total residues will represent approximately 62% of the ADI.