4. Emamectin Benzoate

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Identity

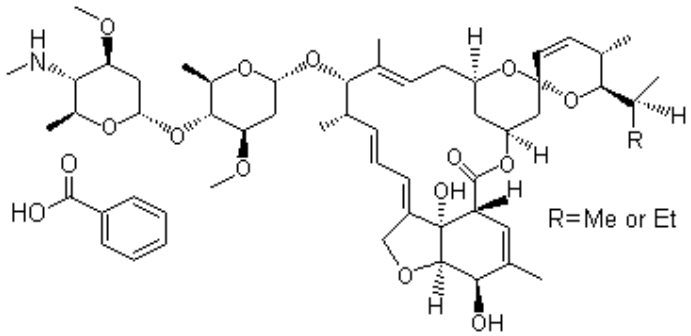
International Non-proprietary Name (INN): emamectin benzoate

Synonyms: AKOS015950774, AB1004837, (4''R)-4''-Deoxy-4''-(methylamino)-avermectin B1 benzoate(salt), emamectin benzoate

IUPAC Names: A mixture containing 90% of (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-6'-[(S)-sec-butyl]-21,24-dihydroxy-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'H-pyran)-12-yl 2,6dideoxy-3-O-methyl-4-O-(2,4,6-trideoxy-3-O-methyl-4-methylamino-alpha-L-lyxo-hexopyranosyl)-alpha-L-arabino-hexopyranoside benzoate
and 10% of (10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-21,24-dihydroxy-6'-isopropyl-5',11,13,22-tetramethyl-2-oxo-3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene-6-spiro-2'-(5',6'-dihydro-2'H-pyran)-12-yl 2,6-dideoxy-3-O-methyl-4-O-(2,4,6-trideoxy-3-O-methyl-4-methylamino-alpha-L-lyxo-hexopyranosyl)-alpha-L-arabino-hexopyranoside benzoate.

Chemical Abstract Service Number: 155569-91-8, formerly 137512-74-4

Structural formula of main components:



SOURCE: http://www.chemblink.com/products/155569-91-8.htm

Molecular formula: B_{1a} component $C_{49}H_{75}NO_{13}C_7H_6O_2$
B_{1b} component $C_{48}H_{73}NO_{13}C_7H_6O_2$

Molecular weight: B_{1a} component: 1008.26 g/mol
B_{1b} component: 994.24 g/mol

Other information on identity and properties

Pure active ingredient:	A mixture of two avermectin homologues: ≥90% of 4′-epimethyamino-4′-deoxyavermectin B1a benzoate (MAB1a), and ≤10% of 4′-epimethyamino-4′-deoxyavermectin B1b benzoate (MAB1b)
Appearance:	The benzoate salt of emamectin, EB, is a white to cream coloured powder.
Melting point:	141–146°C
Solubility:	Water: 24 mg/L (pH 7, 25°C)
Log Ko/w:	5

Residues in food and their evaluation

Conditions of use

Emamectin benzoate is used for the treatment of sea lice in salmon. Major regions of marine salmonid aquaculture activity worldwide include Japan, the east and west coasts of Canada, the northeastern coast of the United States of America, Ireland, Scotland, Norway, Chile, New Zealand and Tasmania (Johnson *et al.*, 2004). Sea lice have not been reported as aquacultural pests in New Zealand or Tasmania. In areas where sea lice infections are common, secondary infections (e.g. with other diseases such as infectious pancreatic necrosis, bacterial kidney disease, and salmonid rickettsial septicaemia) and reduced growth are issues of concern. Secondary infections associated with sea lice infestations have been identified as a serious issue on the east coast of Canada, but not yet on the west coast (Johnson *et al.*, 2004).

Emamectin benzoate received its first registration in Japan in 1998, under the trade name Affirm®. Its use was for the control of lepidopteran pests on leafy vegetables, brassicas and for trunk injection in pine trees to control the pine sawfly (Pesticide Management Advisory Committee, California Department of Pesticide Regulation, www.cdpr.ca.gov). However, emamectin benzoate is not widely used for sea lice control in Japan. Instead, problems associated with sea lice are avoided through rearing of coho salmon, which are less vulnerable to sea lice infestations than Atlantic salmon, coupled with restriction of grow-out periods to about one year.

The emamectin benzoate-based insecticide Proclaim® was granted emergency exemption in Hawaii for control of diamondback moth on horticultural crops and used in 1996 and 1997. Full registration for use was subsequently approved in 1999 (http://www.syngenta-cropprotection.com). In the United States of America, emamectin benzoate is used in terrestrial agriculture to control pests on head lettuce, celery, cauliflower, broccoli, cabbage and other crops. For example, about 260 kg of emamectin benzoate was applied to edible crops in California in 2002 (http://www.pesticideinfo.org; accessed October 2004). Emamectin benzoate has also been widely used in some countries as an anti-fungal agent, sold under the trade name Proclaim®. Overall, emamectin benzoate first came into use in the United States of America and several other countries as a pesticide against terrestrial pests, and its use was shortly thereafter extended to use in finfish aquaculture.

Emamectin benzoate is also formulated as Slice®, which is a trade name for a product developed by Schering-Plough Animal Health (SPAH) now Merck Animal Health. Internationally, Slice® has been developed as an alternative to the use of other sea lice control products, including ivermectin, dichlorvos, azamethiphos, hydrogen peroxide, cyper-

methrin, teflubenzuron and diflubenzuron. Slice® was approved for use in the United Kingdom in 2000. Emamectin benzoate was provided with an "Animal Test Exemption" in 1999 in the UK by the Veterinary Medicine Directorate (VMD) in order to allow field trials to be conducted (Rae, 2000). Prior to this, the European Medicines Evaluation Committee set maximum residue levels (MRLs) for emamectin benzoate in foods intended for human consumption.

The present evaluation was performed on the basis of available published peer-reviewed literature, and monographs prepared by national agencies. Despite the request of the Committee, the sponsor of a marketed authorized emamectin benzoate formulation for sea lice control did not provide the dossier used by national authorities for risk assessment.

Dosage

Emamectin benzoate is administered in Canada as the active ingredient in Slice®, manufactured by the Schering-Plough Animal Health Corporation. The product is supplied as a pre-mix containing 0.2% emamectin benzoate in a 99.8% inert carrier, which comprises 0.01% butylated hydroxyanisole, 2.5% propylene glycol, 47.40% maltodextrin, and corn starch (to 100%) (Scottish Environment Protection Agency, 1999). The pre-mix is coated onto non-medicated fish feed pellets to achieve an intended dose of 50 µg emamectin benzoate per kg of fish biomass per day for seven days. The suggested feeding rate is 0.5% of fish biomass per day. If the feeding rate differs from 0.5% biomass per day, then the concentrations of Slice® in feed must be adjusted accordingly.

The product may be used up to 3 times/year, with a maximum of 5 treatments in any 2-year growth cycle. A withdrawal period of 25 days is required in Canada for emamectin benzoate under its current emergency registration.

Pharmacokinetics and metabolism

Pharmacokinetics in laboratory animals

The following information was obtained from a report issued by the European Medicines Agency (EMEA, 1999). A series of experiments was carried out to determine the fate of the B1a component of emamectin after administration of its benzoate salt to Sprague-Dawley rats. In the first experiment, rats were given a single oral dose of 20 mg/kg bw dual-labelled [^{14}C]/[^{3}H]emamectin B1a and killed 7 days later; the residues in tissues were very similar when based upon [^{14}C] and [^{3}]H radioactivity, indicating stability of the [^{3}H] label. Following administration of a single oral dose of 0.5 mg/kg bw [^{14}C]emamectin B1a, mean peak plasma concentrations of approximately 17 and 21 µg equivalents/kg bw were attained 12 hours and 4 hours after administration, in males and females, respectively. The absolute oral bio-availability of emamectin benzoate 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 within tissues. Seven days after oral administration of 20 mg/kg bw [^{14}C]/[^{3}H]emamectin B1a, residues in tissues ranged from 8 to 2033 µg-equivalents/kg in the following order: most being present in the lung, followed by the gastro-intestinal tract, kidney, liver, fat, bone, muscle, spinal cord and blood, with least residues in the brain. 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 administrated dose was eliminated in faeces and less than 1% in the urine.

Pharmacokinetics in food animals

Salmon

In a pharmacokinetic study of emamectin benzoate in Atlantic salmon, four dose rates (100, 200, 400, 800 µg/kg bw) were administered to smolt fish (average weight 49.7 g) by intra-peritoneal injection (Table 4.1) (Glover *et al.*, 2010). The mean concentrations of residues in muscle and skin were determined by LC/MS 14 days after administration. For all dosages, residue concentrations in skin were considerably higher than for muscle, and a clear relationship between dose and resultant concentration was observed.

Table 4.1. Mean concentration of emamectin residues in muscle and skin of Atlantic salmon 14 days following intra-peritoneal injection (individual dose)

Dosage (µg/kg bw)	Muscle (µg/kg) ±SD	Skin (µg/kg) ±SD
100	26.7 ±7.3	100 ±17.2
200	76.6 ±16.7	159.9 ±50.7
400	166.7 ±43.5	439.8 ±101.1
800	265.2 ±58.4	815.6 ±47.8

SOURCE: Reprinted with permission from Glover, K.A., Samuelsen, O.B., Skilbrei, O.T., Boxaspen, K. & Lunestad, B.T. 2010. Table 1 in Pharmacokinetics of emamectin benzoate administered to Atlantic salmon, *Salmo salar* L. by intra-peritoneal injection. *Journal of Fish Diseases*, 33(2): 183–186. Copyright – 2010 – John Wiley and Sons Ltd.

Following a mean intra-peritoneal administration of 438 µg/kg (range 293–744) emamectin benzoate in smolts, concentrations of emamectin were observed at intervals of 1, 3, 6 and 9 weeks (Table 4.2). Elimination half-lives in muscle and skin were calculated as 11.1 and 10.6 days, respectively.

Table 4.2. Mean concentrations of emamectin residues in muscle and skin of Atlantic salmon over a 9-week period following a single mean intra-peritoneal injection of 438 µg/kg (range: 293–744) of emamectin benzoate

Time (days)	Muscle (µg/kg) ±SD	Skin (µg/kg) ±SD
7	449 ±142	499 ±245
21	158 ±93	185 ±88
42	28 ±7	43 ±13
63	10 ±5	16 ±9

SOURCE: Glover, K.A., Samuelsen, O.B., Skilbrei, O.T., Boxaspen, K. & Lunestad, B.T. 2010. Table 2 in Pharmacokinetics of emamectin benzoate administered to Atlantic salmon, *Salmo salar* L. by intra-peritoneal injection. *Journal of Fish Diseases*, 33(2): 183–186. Copyright – 2010 – John Wiley and Sons Ltd.

Concentrations of emamectin in plasma from salmon that received standard, oral treatments with emamectin benzoate were studied in two fish farms in mid-Norway, with post-smolts put to sea in cages in the autumn of 2005 (Berg and Horsberg, 2009). Samples were collected in the autumn of 2005 and repeated sampling was performed from the same sites in the summer of 2006. The tentative concentration of emamectin benzoate in the medicated feed was 10 mg/kg, resulting in a daily dosage of 50 µg/kg bodyweight at a feeding rate of 5 g medicated feed/kg bodyweight for seven days. Blood samples were collected the day after treatment ended. Blood samples collected from 25 randomly sampled fish at each site in autumn of 2005 and then again in the summer of 2006 were analysed for emamectin by a HPLC method. The overall median concentration of emamectin B1a in plasma for the results from all sampling dates was 116 ng/ml, with concentrations ranging from 6 ng/ml in autumn, 2005, to 440 ng/ml in summer, 2006.

When tested statistically using Friedman's ANOVA, there were significant differences in the emamectin plasma concentrations among fish from three cages at one of the farms, both in the autumn of 2005 and in summer 2006. At the second farm, no significant differences among three cages could be demonstrated in 2005 or in 2006. When pooling the results from all three cages, no significant difference could be demonstrated between the samples collected in autumn 2005 and summer 2006 at the one farm, whereas at the second farm, a highly significant difference was demonstrated between seasons. When looking at the pooled results from each site, no significant difference was detected between the two sites in 2005, whereas in 2006 there was a highly significant difference.

Cod

Emamectin benzoate was studied in cod, *Gadus morhua*, held in seawater at 9°C and weighing 100–200 g (Samuelsen, 2010). Concentrations of emamectin B1a were determined in plasma collected from treated fish after intravenous (i.v.) injection (50 µg/kg bw) and in plasma, muscle and skin following single oral (through stomach intubation, 50 µg/kg bw) administration. Following i.v. injection, the plasma drug concentration-time profile showed two distinct phases. The plasma distribution half-life ($t_{½}\alpha$) was estimated to be 2.5 h, the elimination half-life ($t_{½}\beta$) as 216 h, the total body clearance (Cl_T) as 0.0059 L/kg/h and mean residence time (MRT) as 385 h. The volume of distribution at steady state, VD_{ss}, was calculated to be 1.839 L/kg. Following *per os* administration, the peak plasma concentration (C_{max}) was 15 ng/ml, the time to peak plasma concentration (T_{max}) was 89 h and $t_{½}\beta$ was 180 h. The highest concentration in muscle (21 µg/kg) was measured after 7 days, and $t_{½}\beta$ was calculated to be 247 h. For skin, a peak concentration of 28 µg/kg at 3 days was observed and a $t_{½}\beta$ of 235 h was determined. The bio-availability following *per os* administration was calculated to be 38%.

Metabolism in laboratory animals

Rats

In rats, approximately 80% of the radiolabelled material in faeces and in tissues was unchanged emamectin B1a (EMEA, 1999). 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 quantity of this metabolite in faeces increased with time post-dosing. In faeces, this metabolite represented approximately 1 to 2% of the radioactivity on Day 1 after dosing, but 18 to 19% on Day 7 after dosing. The percentage of this metabolite found in faeces was independent of the dose administered, the route of administration or the sex of the animal.

Metabolism in food producing animals

Salmon

One hundred and twenty-two unsexed Atlantic salmon, averaging 1.3 kg bw, were used in a metabolism study (Kim-Kang *et al.*, 2004). The fish were maintained at 5°C in two identical 1800 L tanks (A and B) of re-circulating seawater connected to a single reservoir. After an acclimatization period of 14 days, 100 fish were given [^{3}H]emamectin benzoate formulated in feed to provide a nominal dose of 50 µg/kg of live weight/day for 7 consecutive days, based on a feeding rate of 0.4% bodyweight per day and the total biomass in each tank. The entire daily medicated feed ration was offered to all fish in each tank over a 30-minute period and any uneaten feed then removed. The remaining 22 fish served as unmedicated controls, 4 being removed before dosing and 18 being placed in the tanks with the dosed fish after dosing was completed.

The treated feed was prepared by mixing a feed pre-mix containing the [^{3}H]emamectin benzoate, sieved Atlantic salmon pellet feed, and fish oil. The feed pre-mix used for the treated feed preparation was of similar composition to commercial Slice®. The actual daily dose of the drug received was approximately 33 µg/kg per fish. Analysis of the treated feed before and after dosing confirmed that the [^{3}H]emamectin B1a had a radiopurity of 98%. Groups of 10 dosed fish were euthanized at 3, 12, 24 and 72 h and at 7, 15, 30, 45, 60 and 90 days post-dose. Faeces were collected daily from the tanks, beginning just prior to dosing to 90 days post-final dose. Control fish (n = 4) were removed 96 h prior to initial dosing. After weighing, mucus was collected from both flanks. Samples (n = 5) of liver, kidney, gut contents, muscle, skin and intact skin-with-muscle were pooled by matrix and post-dose interval for metabolite profiling at 2 h, Day 7, Day 15, Day 45 and Day 90. Samples were analysed using a HPLC system combined with a UV detector and liquid cell radioactivity

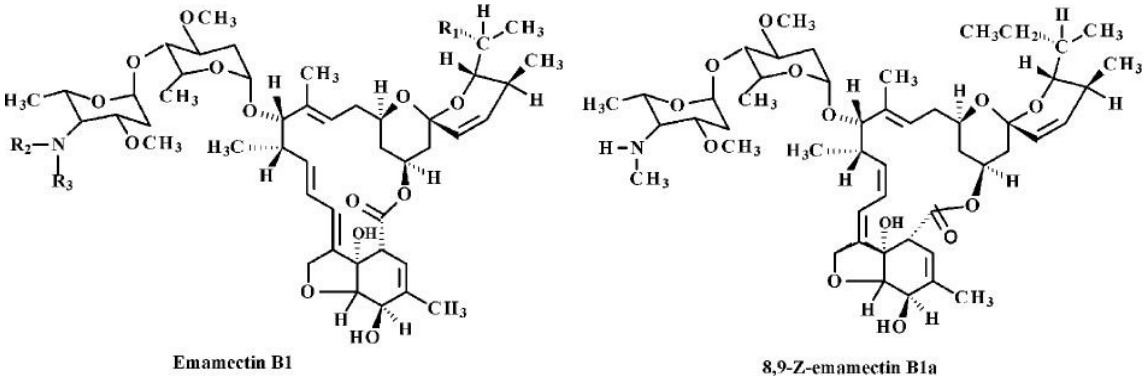
monitoring, or via fraction collection followed by liquid scintillation counting (LSC).

Muscle, skin and intact skin-with-muscle tissues containing incurred residues from the 3-, 12-, 24- and 72-h, and 7-, 15- and 30-day withdrawal times were analysed by HPLC with fluorescence detection (HPLC/FL). Methanolic extractions of pooled tissues (liver, kidney, muscle, skin and intact skin-with-muscle), as well as pooled gut contents and faeces samples, were prepared for selected intervals. The extractability of [^{3}H] residues in each matrix at all time-points was excellent: >99% (liver), >98% (kidney, intact skin-with-muscle), >97% (muscle, gut contents), >94% (skin and faeces). Recovery of radioactivity for the method was excellent, ranging from 98.31% for faeces to >100% (104–115%) for other matrices.

The metabolic profiles of emamectin residues in tissues, gut contents and faeces were established by HPLC using emamectin B1a and four metabolite standards: desmethylemamectin B1a; 8,9-Zemamectin B1a; N-desmethyl-N-formylemamectin B1a; and N-formylemamectin B1a. The final extracts of pooled samples from each time-point (12 h through Day 90) were analysed by HPLC. The HPLC conditions used were sufficient to afford good baseline separation of the components identified in Figure 4.1. The proportion of emamectin B1a in all tissues generally decreased from 98 to 100% of TRR at 12 h post-final dose to 81–89% by Day 90. Lesser amounts of [^{3}H] component 7, which co-chromatographed with desmethylemamectin B1a, were observed in nearly all tissue extracts, increasing from 0 to 1% TRR at 12 h post-final dose to 11–17% TRR by Day 90. Another component ([^{3}H] component 11, N-formylemamectin B1a) was inconsistently observed at low levels (<2% TRR) in several extracts. No other significant residues were observed in tissues. HPLC analysis of 12-h and 90-day gut contents indicated that emamectin B1a and its desmethyl metabolite were the primary residues, although several minor residues were also present. HPLC analysis of faeces collected during dosing showed emamectin B1a as essentially the only component, whereas analysis of pooled faeces collected from 0 to 7 days post-final dose indicated the presence of several minor components of 2–10% TRR each in addition to emamectin B1a at 57% TRR.

Figure 4.1. Structures of emamectin and metabolites

(Reprinted with permission from Kim-Kang, H., Bova, A., Crouch, L.S., Wislocki, P.G., Robinson, R.A. & Wu, J. 2004. Figure 2 in: Tissue distribution, metabolism, and residue depletion study in Atlantic salmon following oral administration of [^{3}H]emamectin benzoate. *Journal of Agricultural and Food Chemistry*, 52(7): 2108–2118. Copyright – 2004 – American Chemical Society.



Compound ID	R_1	R_2	R_3
8,9-Z-emamectin B1a	CH_2CH_3	H	CH_3
N-formyl emamectin B1	CH_2CH_3 for B1a, CH_3 for B1b	CH=O	CH_3
N-desmethyl, N-formyl-emamectin B1	CH_2CH_3 for B1a, CH_3 for B1b	CH=O	H
N-desmethyl-emamectin B1	CH_2CH_3 for B1a, CH_3 for B1b	H	H

Tissue residue depletion studies

Radiolabelled residue depletion studies

Salmon

Information on the relationship between total radiolabelled residues and marker residue was also obtained from the metabolism study in which Atlantic salmon (1.3 kg bw) maintained in tanks of seawater at 5 ±1°C were dosed with [^{3}H]emamectin B1 benzoate in feed at a nominal rate of 50 µg of emamectin benzoate/kg/day for 7 consecutive days (Kim-Kang *et al.*, 2004). Tissues, blood and bile were collected from 10 fish each at 3 and 12 h and at 1, 3, 7, 15, 30, 45, 60 and 90 days post-final dose. Samples of blood, liver, kidney, skin and muscle were collected for total radioactivity residue (TRR) determination by combustion/LSC using biological sample oxidizers analysis. Emamectin B1a concentrations in muscle and skin were quantified using a validated HPLC/FL method based on extraction and derivatization of emamectin B1a. The LOQ for both tissues was 40 µg/kg and the LODs were 2.6 µg/kg and 3.3 µg/kg for muscle and for skin, respectively.

The highest TRR concentration in tissues was found in kidney (306 ±73 µg/kg on day 15, declining to 144 ±44 µg/kg by Day 90). TRR concentrations in muscle varied from 53 ±19 to 65 ±19 µg/kg in the first 72 h post-dose, declining to 19 ±5 µg/kg by Day 90, whereas TRR levels in skin ranged from 69 ±27 to 93 ±34 µg/kg during the first 72 h post-dose, declining to 36 ±10 µg/kg by Day 90. For muscle-with-skin, TRR levels varied from 55 ±19 to 64 ±20 µg/kg in the first 72 h post-dose, decreasing to 20 ±6 µg/kg by Day 90. TRR levels in plasma were modest, ranging from 119 ±40 µg/kg at 72 h, to 30 ±10 µg/kg by Day 90, whereas those in mucus amounted to 10 ±7 µg/kg through the entire testing interval. Residues in bone were also low, ranging from 7 ±2 µg/kg at 72 h to 2 ±1 µg/kg by Day 90. The TRR levels in bile and gut contents ranged from 638 ±121 µg/kg (bile, 72 h) to 149 ±53 µg/kg and from 669 ±140 µg/kg (gut content, 3 h) to 156 ±62 µg/kg by Day 90.

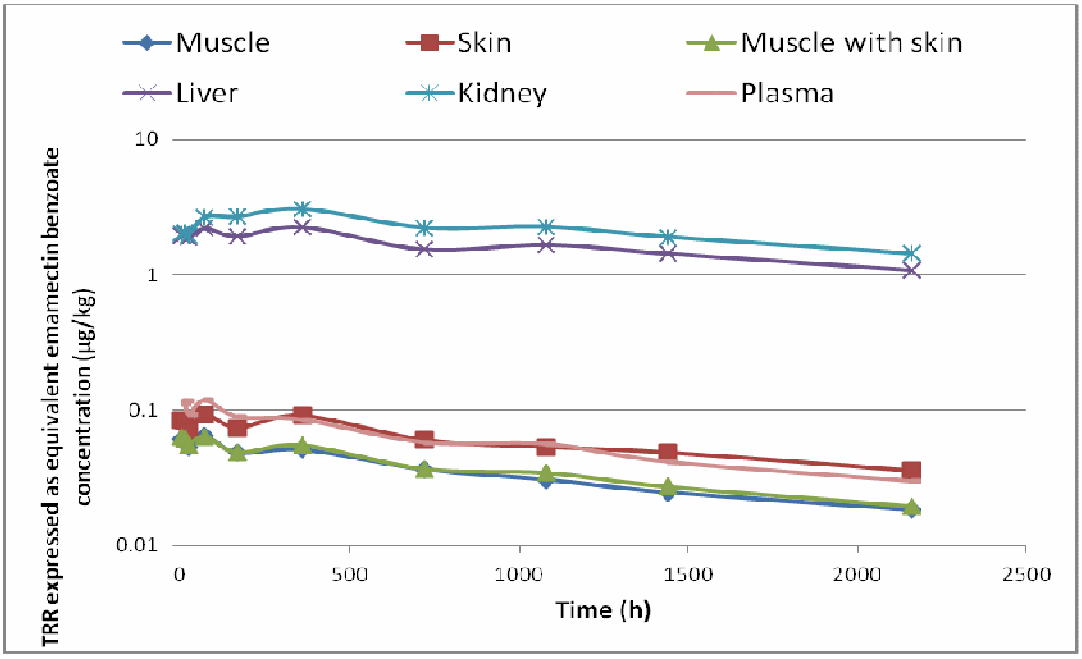


Figure 4.2. TRR depletion profiles in plasma muscle, skin, muscle-and-skin, liver and kidney of salmon dosed at a nominal rate of 50 µg of [^{3}H]emamectin B1 benzoate/kg/day in feed for 7 consecutive days.

The distribution of total residue was greatest in liver and kidney, with 28–34 and 22–35% of the total residue found in these two organs, respectively. Muscle (13–20%) and intact skin-with-muscle (15–22%) also contained a sizable portion of the total emamectin residue. Bile and gut contents contained a small proportion of the total emamectin residue (1–5%), whereas skin, bone, mucus and plasma each contained only 1% or less. The residue components identified in liver, kidney, muscle, and skin samples pooled by post-dose interval were emamectin B1a (81–100% TRR) and desmethylemamectin B1a (0–17% TRR) with N-formylemamectin B1a seen in trace amounts (<2%) in some muscle samples. The emamectin B1a concentration in individual samples of skin and muscle as determined using HPLC/FL was below 85 µg/kg in all samples analysed (3 h to 30 days post-dose).

Emamectin B1a in muscle ranged from 32 to 67 µg/kg at 3 h, from 36 to 58 µg/kg at 12 h, from 18 to 60 µg/kg at 24 h, from 24 to 64 µg/kg at 72 h, from 12 to 55 µg/kg at 7 days, from 19 to 48 µg/kg at 15 days, and from 13 to 39 µg/kg at 30 days. The mean ratio of the marker to total radioactive residue in muscle was rather constant, ranging from 0.66 to 0.73. Emamectin B1a in skin ranged from 37 to 84 µg/kg at 3 h, from 28 to 68 µg/kg at 12 h, from 24 to 74 µg/kg at 24 h, from 23 to 84 µg/kg at 72 h, from 15 to 48 µg/kg at 7 days, from 31 to 61 µg/kg at 15 days, and from 16 to 59 µg/kg at 30 days. The mean ratio of the marker to total residue in skin was also rather constant, ranging from 0.56 to 0.66 (Kim-Kang *et al.*, 2004[1]).

The ratio of emamectin B1a, as determined by HPLC/FL in the determinative assay relative to the TRR is significantly less than 1, despite the high extractability of [^{3}H] residues and the high proportion of [^{3}H]emamectin B1a as determined by HPLC-radiometry. This difference is accounted for by the expression of TRR in the study as emamectin B1 benzoate equivalents, whereas emamectin B1 benzoate is only 80% emamectin B1a free base and recoveries of 83–91% were obtained in the quantitative assay. After correction, the ratio of the mean concentration of the marker residue emamectin B1a to that of the total residue was calculated as 0.9 for muscle and fillet (muscle+skin), and 0.8 for skin.

Salmon

The tissue distribution of [^{3}H]emamectin benzoate after a single oral dose in Atlantic salmon (Sevatdal *et al.*, 2005) was investigated by means of whole-body autoradiography and scintillation counting. The distribution study demonstrated a high quantity of radioactivity in mucous membranes (gastrointestinal tract, gills) throughout the observation period (56 days). Activity was also high in the epiphysis, hypophysis and olfactory rosette throughout the study. The highest activity was observed in the bile, indicating this to be an important route for excretion. The distribution study confirmed the results from the elimination study with respect to concentrations in blood, skin mucous and muscle.

Residue depletion studies with unlabelled drug

Salmon

A study was conducted to investigate the content of emamectin in blood, mucus and muscle following field administration of the recommended dose of emamectin (50 µg/kg bw, daily for 7 days) to salmon with an average weight of 135 g at the start of the treatment (Sevatdal *et al.*, 2005). Salinity varied between 3.2 and 3.4% during the trial period. The study was

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[1] The reviewers noted some apparent discrepancies in Table 7 in Kim-Kang *et al.*, 2004. The concentrations reported for emamectin B1a residues in skin for the group of fish 41–50 (Day 15) and 51–60 (Day 30) appear to have been transposed from the preceding table (i.e. Table 6 in Kim-Kang *et al.*, 2004). The values reported for fish 42, 44, 46, 48 and 50 prior to the group 41–50 in the table may represent the concentrations of emamectin B1a found in skin of these fish, as they differ from the values reported in the preceding table. The Ratio of marker:TRRs reported in Table 7 of Kim-Kang *et al.*, 2004, for emamectin residues in skin at Days 15 and 30 are not consistent with the ratios reported in the text of the original paper. These discrepancies did not, however, affect the ratio MR:TRR determined by the Committee.

performed in the field at ambient water temperatures, which varied between 15°C (June) and 19°C (August). Blood, muscle and mucus samples were collected before the treatment started (Day 0) and on Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 after start of treatment. After derivatization of the samples with 1-methylimidazole and trifluoroacetic anhydride (60°C, 90 min), the concentrations of emamectin B1a in plasma, mucus and muscle were determined by high-performance liquid chromatography using a fluorescence detector. Maximum concentrations of 128, 105 and 68 µg/kg of emamectin B1a were reached in blood, mucus and muscle, respectively, on Day 7 (the last day of treatment). From Day 7, the concentration of emamectin B1a in the blood declined until the concentration was less than the limit of detection (1 µg/kg) on Day 77. The concentration was significantly higher in mucus compared with plasma (P <0.05) except on Days 7 and 21. The concentration of emamectin B1a decreased gradually from the end of treatment (Day 7) to Day 70, with biological half-lives of 9.2, 10.0 and 11.3 days in muscle, plasma and mucus, respectively (Table 4.3).

Table 4.3. Average concentration (µg/kg) of emamectin B1a in blood, mucus and muscle of Atlantic salmon after administration of a recommended dose (50 µg emamectin benzoate per kg daily for 7 days) (n = 10 for each sampling)

Day	Concentration of emamectin B1a (SD) (µg/kg)		
	Mucus	Blood	Muscle
0	0	0	0
7	104.6 (67.5)	128.3 (43.5)	74.8 (28.4)
14	74.1 (27.3)	39.7 (9.1)	–
21	42.7 (41)	27.9 (13.5)	20.9 (9.9)
28	37.6 (20.9)	13.1 (8.2)	–
35	27.4 (9.3)	8.6 (3.6)	8.5 (2.0)
42	10.5 (5.7)	4.2 (3.2)	–
49	6.0 (2.2)	3.5 (1.4)	3.2 (1.2)
56	4.9 (2.3)	1.7 (1.3)	–
63	3.0 (1.2)	0	0
70	3.5 (0.5)	1.0 (0.5)	–
77	1.4 (0.7)		

SOURCE: Reprinted with permission from Sevatdal, S., Magnusson, Å., Ingebrigtsen, K., Haldorsen, R. & Horsberg, T.E. 2005. Table 1 in: Distribution of emamectin benzoate in Atlantic salmon (*Salmo salar* L.). *Journal of Veterinary Pharmacology and Therapeutics*, 28(2): 101–107. Copyright – 2005 – John Wiley and Sons Ltd.

The depletion of emamectin B1a in the edible tissues of Atlantic salmon (*Salmo salar*) was studied at two inclusion rates of emamectin benzoate (Slice®) in commercially prepared diets (Whyte *et al.*, 2011). Fish were maintained in tanks supplied by flow-through, temperature-controlled natural seawater at 10.08 ±0.26°C, and administered a medicated diet containing a nominal dose rate of emamectin benzoate at either 50 µg/kg bw per day (the recommended manufacturer's dose), or 100 µg/kg bw per day, for seven consecutive days. Individual variability in concentrations of emamectin B1a in muscle and skin tissues was high and considered related to the hierarchal effects of feeding. Emamectin B1a residues were determined in muscle fillet and skin samples at intervals from 1 to 45 days post-feeding in the single-only dose study, and 1 to 20 days post-feeding in the single–double dose comparison study. Mean concentrations of emamectin B1a ranged from 60.5 to 7.3 µg/kg in the muscle and 199.7 to 28.1 µg/kg in the skin for the single-only study, and in the single–double comparison study the range was 57.5 to 25.8 ng/g in the muscle of the single dose and 96.8 to 45.6 µg/kg in the muscle of the double dose. The maximum residue limits allowed by Health Canada of 100 µg/kg and 1000 µg/kg were never detected in the muscle and skin, respectively, for the recommended dosage of 50 µg/kg, whereas 28.6% of the fish receiving the double dose of 100 µg/kg were observed to have residue concentrations greater than 100 µg/kg for muscle, ranging from Day 1 through Day 20 post-medication (Table 4.4).

Table 4.4. Concentration of emamectin B1a in muscle and skin of Atlantic salmon (µg/kg) after administration of different doses of emamectin benzoate.

Sampling interval post-medication	Dosage			
	50 µg/kg for 7 days		50 µg/kg for 7 days	100 µg/kg for 7 days
	Emamectin B1a concentration (µg/kg)			
	Muscle	Skin	Muscle	Muscle
Control	0	0	0	0
1 day	60.5 ±28.5	199.7 ±115.9	57.5 ±21.3	96.8 ±84.6
Internal control	0	0	Not applicable	
3 days	55.4 ±32.8	153.3 ±65.8	33.0 ±36.9	89.8 ±49.6
Internal control	0	2.42 ±2.1	Not applicable	
Off-feed	35.0 ±13.0	121.1 ±83.2	Not applicable	
5 days	36.5 ±19.4	219.6 ±121.2	39.5 ±28.2	79.8 ±52.0
Internal control	0	2.37 ±2.1	Not applicable	
Off-feed	31.5 ±20.4	99.4 ±63.6	Not applicable	
10 days	32.3 ±17.0	143.8 ±90.1	25.6 ±17.8	58.5 ±44.8
Internal control	0	1.47 ±1.3	Not applicable	
15 days	24.9 ±15.7	104.1 ±55.0	21.3 ±10.4	37.0 ±35.1
Internal control	0	0	Not applicable	
20 days	Not applicable		25.8 ±17.3	45.6 ±33.5
30 days	12.9 ±7.4	69.6 ±30.9	Not applicable	
Internal control	0	2.01 ±1.7	Not applicable	
45 days	7.3 ±3.0	28.1 ±13.0	Not applicable	
Internal control	0	0.89 ±1.3	Not applicable	

SOURCE: Reprinted with permission from Whyte, S.K., Westcott, J.D., Byrne, P. & Hammell, K.L. 2011. Table 3 in: Comparison of the depletion of emamectin benzoate (Slice®) residues from skeletal muscle and skin of Atlantic salmon (*Salmo salar*) for multiple dietary dose regimens at 10°C. *Aquaculture*, 315(3-4): 228–235. Copyright – 2011 – Elsevier B.V.

In another study, juvenile Atlantic salmon with an initial mean weight of 132 g were experimentally medicated by a standard seven-day emamectin benzoate treatment of 50 µg/kg bw (Olsvik *et al.*, 2008). The concentrations of emamectin B1a in liver, muscle and skin were determined, by LC-MS/MS (LOQ = 5 µg/kg, LOD = 2.5 µg/kg), in samples collected at Days 7, 14 and 35 after the start of treatment. At Day 7, the mean concentration of emamectin B1a in samples of liver was 33 µg/kg, whereas the mean concentrations in muscle were around 1 µg/kg. The skin did not contain emamectin B1a in concentrations above the level of detection at Day 7. At Day 14, the mean concentrations in liver, muscle and skin were 9002, 81 and 369 µg/kg, respectively. The corresponding mean concentrations at Day 35 were 4902, 34 and 258 µg/kg, respectively.

Trout

The depletion of emamectin B1a in the edible tissues of rainbow trout (*Oncorhynchus mykiss*) was studied at two temperatures following treatment with emamectin benzoate in feed using a standard seven-day treatment of 50 µg/kg bw (Roy *et al.*, 2006). Fish approaching market size (400–1500 g) were held in tanks supplied with temperature-controlled seawater at 6 ±1°C (cold water) or 15 ±1°C (warm water). In each study, the medicated group was offered feed containing emamectin benzoate at a nominal dose rate of 50 µg/kg bw fish/day for 7 days and the control group was offered un-medicated feed. Actual dose rates, calculated from growth rate and feed consumption data, and measured emamectin benzoate concentrations in feed, were 88.6% nominal in the cold water study (96.6% adjusted for feed assay recovery) and 96.8% nominal in the warm water study (105.1% adjusted for feed assay recovery).

Concentrations of emamectin B1a were determined in fillet samples (muscle and skin in natural proportion) collected at intervals from 6 h to 77 days post-treatment in the cold water study, and 6 h to 49 days post-treatment in the warm water study. In the cold water study, mean emamectin B1a residues ranged from 81.8 ±44.5 µg/kg at 1 day post-treatment (102.3 ±55.7 µg/kg adjusted for recovery) to 13.7 ±10.5 µg/kg at 77 days post-treatment (17.2 ±13.1 µg/kg). In the warm water study, mean residue concentrations ranged from 64.5 ±50.3 µg/kg at 6 h post-treatment (80.7 ±62.9 µg/kg adjusted for recovery) to 1.6 ±1.6 µg/kg at 49 days post-treatment (2.0 ±2.0 µg/kg). In the cold water study, residues in skin and muscle were also determined separately (Tables 4.5–4.8). On average, emamectin B1a concentrations in skin were approximately 1.8 times higher than in muscle. Measured residue levels ranged widely and no detectable residues were found in at least a few individual fish at all time-points. This high variability was considered to be due to differences in medicated feed consumption within the experimental population. Depletion of emamectin was faster at 15°C than at 6°C. In both studies the depletion curve showed a small secondary peak at around 90 degree-days. This observation is consistent with recirculation of the compound from a body store.

Table 4.5. Residues of emamectin B1a at each time-point following treatment, in fillet (muscle + skin in natural proportion) from trout which received nominally 50 µg/kg bw emamectin benzoate for 7 days and which were maintained at 6°C for the duration of the experiment.

Days post-treatment	Measured concentration (µg/kg)			Recovery adjusted concentration (µg/kg)		
	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
0.25	<1.6	99.3	54.4 ±28.4	<2.0	124	68.0 ±35.5
1	<1.6	142	81.8 ±44.5	<2.0	178	102.3 ±55.7
3	<1.6	112	48.4 ±34.3	<2.0	140	60.5 ±42.9
7	<1.6	125	56.8 ±42.8	<2.0	156	70.9 ±53.4
21	<1.6	99.4	32.1 ±30.6	<2.0	124	40.2 ±38.2
35	<1.6	96.3	34.1 ±35.2	<2.0	120	42.6 ±43.9
56	<1.6	77.1	17.9 ±21.0	<2.0	96.3	22.3 ±26.3
77	<1.6	36.2	13.7 ±10.5	<2.0	45.2	17.2 ±13.1

NOTES: Mean ±SD calculated from 15 fish. Individual measured values reported as ND (not detectable assigned value of LOD/2; individual measured values reported as NQ (not quantifiable) assigned value of LOQ/2. LOD = 1.6 µg/kg, LOQ = 20.6 µg/kg. SOURCE: Reprinted with permission from Roy, W.J., Gillan, N., Crouch, L., Parker, R., Rodger, H. & Endris, R. 2006. Table 3 in: Depletion of emamectin residues following oral administration to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 259(1-4): 6–16. Copyright – 2006 – Elsevier B.V.

Table 4.6. Residues of emamectin B1a at each time-point following treatment, in skin of trout that received nominally 50 µg/kg bw emamectin benzoate for 7 days and which were maintained at 6°C

Days post-treatment	Measured concentration (µg/kg)			Recovery adjusted concentration (µg/kg)		
	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
0.25	<2.1	167	86.8 ±53.9	<2.5	199	103.4 ±64.1
1	<2.1	337	120.6 ±87.4	<2.5	402	143.6 ±104.3
3	<2.1	165	75.0 ±53.9	<2.5	196	89.3 ±64.2
7	<2.1	169	88.1 ±58.7	<2.5	201	105.1 ±70.0
21	<2.1	198	54.4 ±55.6	<2.5	235	64.8 ±66.1
35	<2.1	175	51.8 ±54.5	<2.5	208	61.6 ±64.8
56	<2.1	113	25.0 ±30.6	<2.5	135	29.9 ±36.5
77	<2.1	50.1	20.3 ±13.2	<2.5	59.6	24.2 ±15.8

NOTES: Mean ±SD calculated from 15 fish. Individual measured values reported as ND (not detectable) assigned value of LOD/2; individual measured values reported as NQ (not quantifiable) assigned value of 4.68 µg/kg. LOD= 2.1 µg/kg, LOQ = 20.7 µg/kg. SOURCE: Reprinted with permission from Roy, W.J., Gillan, N., Crouch, L., Parker, R., Rodger, H. & Endris, R. 2006. Table 5 in: Depletion of emamectin residues following oral administration to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 259(1-4): 6–16. Copyright – 2006 – Elsevier B.V.

Table 4.7. Residues of emamectin B1a at each time-point following treatment, in muscle of trout that received nominally 50 µg/kg bw emamectin benzoate for 7 days and which were maintained at 6°C

Days post-treatment	Measured concentration (µg/kg)			Recovery adjusted concentration (µg/kg)		
	Min	Max	Mean ±SD	Min	Max	Mean ±SD
0.25	<1.6	103	52.8 ±28.5	<1.9	121	62.1±33.5
1	<1.6	123	63.9 ±35.2	<1.9	145	75.2 ±41.5
3	<1.6	118	48.0 ±36.5	<1.9	139	56.5 ±43.0
7	<1.6	120	53.3 ±42.0	<1.9	141	62.8 ±49.4
21	<1.6	86.1	23.4 ±26.9	<1.9	101	27.5 ±31.6
35	<1.6	86.2	25.4 ±27.4	<1.9	101	29.9 ±32.2
56	<1.6	51.5	13.4 ±13.6	<1.9	60.6	15.9 ±15.9
77	<1.6	34.1	13.7 ±10.2	<1.9	40.1	16.2 ±12.0

NOTES: Mean ±SD calculated from 15 fish. Individual measured values reported as ND (not detectable) assigned value of LOD/2; individual measured values reported as NQ (not quantifiable) assigned value of 4.7 µg/kg. LOD = 1.6 µg/kg, LOQ = 20.6 µg/kg. SOURCE: Reprinted with permission from Roy, W.J., Gillan, N., Crouch, L., Parker, R., Rodger, H. & Endris, R. 2006. Table 4 in: Depletion of emamectin residues following oral administration to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 259(1-4): 6–16. Copyright – 2006 – Elsevier B.V.

Table 4.8. Residues of emamectin B1a at each time-point following treatment, in fillet (muscle + skin in natural proportion) from trout which received nominally 50 µg/kg bw emamectin benzoate for 7 days and which were maintained at 15°C at each time-point following treatment.

Days post-treatment	Measured concentration (µg/kg)			Recovery adjusted concentration (µg/kg)		
	Min.	Max.	Mean ±SD	Min.	Max.	Mean ±SD
0.25	<1.6	130	64.5 ±50.3	<2.0	162	80.5 ±62.8
1	<1.6	106	45.1 ±31.8	<2.0	132	56.4 ±39.7
3	<1.6	150	43.6 ±57.3	<2.0	188	54.6 ±71.8
7	<1.6	81.3	17.9 ±31.1	<2.0	102	22.4 ±39.0
14	<1.6	47.1	24.7 ±20.8	<2.0	158.9	30.8 ±26.0
21	<1.6	24.1	8.7 ±9.4	<2.0	30.1	10.9 ±11.7
35	<1.6	<9.35	3.1 ±2.0	<2.0	<11.7	3.9 ±2.5
49	<1.6	<9.35	2.0 ±1.9	<2.0	<11.7	2.5 ±2.3

NOTES: Mean ±SD calculated from 15 fish. Individual measured values reported as ND (not detectable) assigned value of LOD/2; individual measured values reported as NQ (not quantifiable) assigned value of LOQ/2. LOD = 1.6 µg/kg, LOQ = 20.6 µg/kg. SOURCE: Reprinted with permission from Roy, W.J., Gillan, N., Crouch, L., Parker, R., Rodger, H. & Endris, R. 2006. Table 8 in: Depletion of emamectin residues following oral administration to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 259(1-4): 6–16. Copyright – 2006 – Elsevier B.V.

Methods of analysis for residues in tissues

An analytical method was developed and validated to determine residue concentration of emamectin B1a in muscle, skin, and intact muscle+skin in natural proportion from Atlantic salmon (Kim-Kang *et al.*, 2004). The method is based on cleanup of an ethyl acetate extract of tissue on a propylsulfonic acid solid phase extraction cartridge, followed by derivatization with trifloroacectic anhydride in the presence of N-methylimidazole. The amount of derivatized emamectin B1a present is determined using reversed phase HPLC/FL. Calibration curves were obtained with fortified tissue over a range of 50–800 µg/kg. The LODs were 2.6, 8.3 and 3.8 µg/kg as emamectin B1a for muscle, skin and intact muscle+skin, respectively. The LOQs were all at 50 ng/g for muscle, skin and intact muscle+skin. Recoveries were 94.4 ±6.89% for muscle, 88.4 ±5.35% for skin, 88.0 ±3.73% for intact muscle+skin.

Another HPLC/FL method for determining residues of emamectin and ivermectin in fish tissues has been developed in which residues are extracted with acetonitrile and cleaned up on a C18 solid-phase extraction column (Van De Riet *et al.*, 2001). Extracts are derivatized with 1-methylimidazole and trifluoroacetic anhydride, and the components are determined on a C18 reversed phase column with fluorescence detection (excitation: 365 nm; emission: 470 nm). The mobile phase is 94% acetonitrile-water run isocratically. Calibration curves were linear between 1 and 32 ng injected for both emamectin and ivermectin. The limit of detection for both analytes was 0.5 µg/kg, with a limit of quantitation of 1.5 µg/kg. Recoveries of emamectin and ivermectin added to salmon muscle averaged 96 ±9% and 86 ±6%, respectively, at levels between 5 and 80 µg/kg.

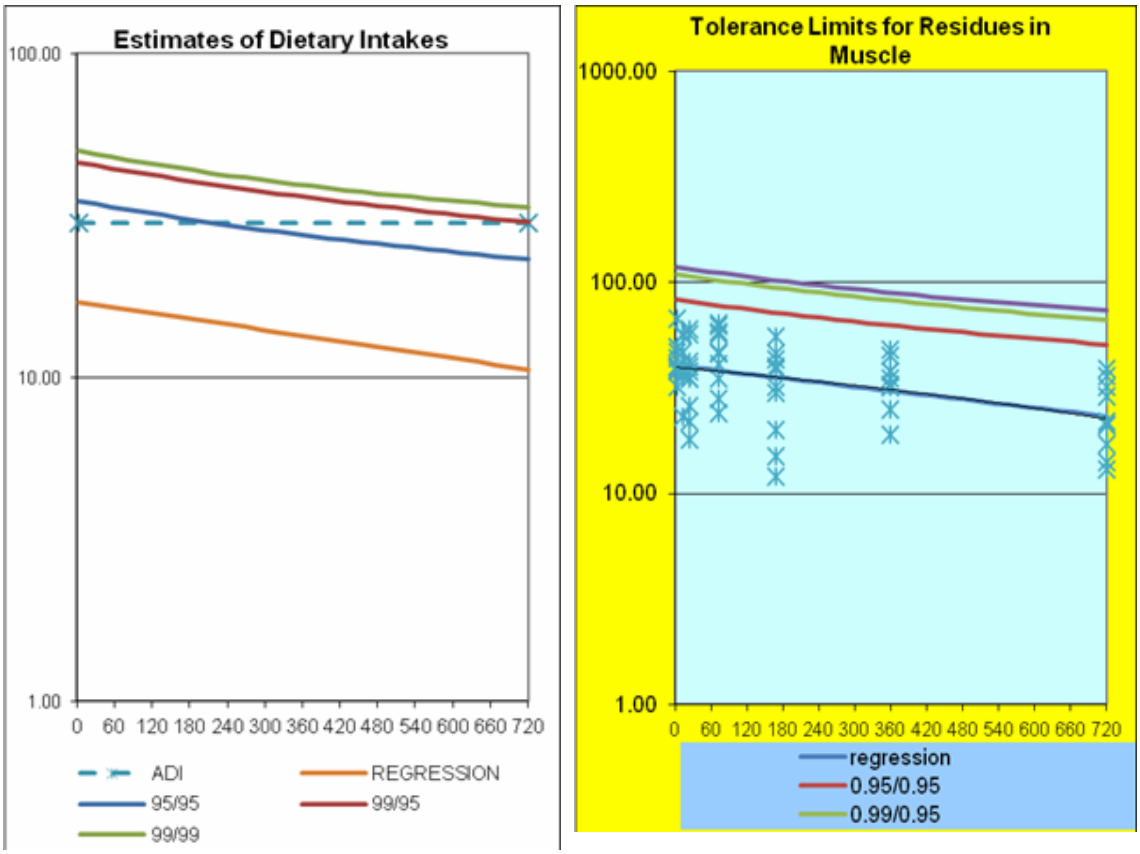

Figure 4.3. Estimated dietary intakes (expressed as emamectin benzoate equivalents) and tolerance limits in salmon muscle expressed as emamectin B1a

Appraisal

Emamectin has not been previously reviewed by the Committee. Emamectin benzoate is a semi-synthetic avermectin that is registered for aquaculture use in the treatment of salmon and trout at a maximum recommended dose of 50 µg/kg fish/day for 7 days, administered through medicated feed, for control of infestation by sea lice.

A radiolabelled study in salmon demonstrated that emamectin B1a is the marker residue and that it remains predominantly unmetabolized (Kim-Kang *et al.*, 2004). Over the time interval between the last administration and 70 days post-administration, the ratio between the marker residue and the total radioactive residue (expressed as emamectin benzoate equivalents) was stable in muscle and skin, at 65 ±7% for both tissues. After correction, the ratio of the mean concentration of the marker residue emamectin B1a to that of the total residue was calculated as 0.9 for muscle and fillet (muscle+skin), and 0.8 for skin. Residues

decline slowly in fish and terminal half-lives are dependent on environmental conditions.

The recommended MRLs of 100 µg/kg of emamectin in muscle and fillet (muscle+skin) are based on the upper limit of the one-sided 99% confidence interval over the 99th percentile ("99/99 tolerance limit") for the 10-day post-treatment of the radiolabelled depletion curve. This highest value 99/99 instead of 95/95 was chosen by the committee to cover the uncertainty associated to the high terminal half-life and the variation of kinetics in fish in relation to life conditions.

Residue data were obtained using a validated analytical method to quantify emamectin B1a in tissue. Residue data from two independent studies were also provided for trout administered unlabelled emamectin benzoate at the approved dose rate. Median concentrations in muscle and fillet reported in trout were in the same range as those observed in salmon (Roy *et al.,* 2006). Trout and salmon belong to the sub-family of Salmonidae.

Maximum Residue Limits

In recommending MRLs for emamectin B1a in salmon and trout, the Committee considered the following factors:

- Emamectin benzoate is authorized for use in salmon and trout. For salmon, the maximum recommended dose is 50 µg/kg fish per day for 7 days, administered through medicated feed.
- An ADI for emamectin benzoate of 0–0.5 µg/kg bw was established by the Committee, corresponding to an upper bound of acceptable intake of 30 µg/day for a 60 kg person.
- Emamectin B1a is predominantly unmetabolized.
- Emamectin B1a is the marker residue in tissues.
- The ratio of the concentration of marker residue to the concentration of total residue is 0.9 in muscle and fillet of salmon.
- Residue data were provided using a validated analytical method to quantify emamectin B1a in tissue.
- Residue data in trout were available.
- A validated analytical method for the determination of emamectin B1a in edible tissue of salmon and trout is available and may be used for monitoring purposes.

MRLs were calculated on the basis of the upper limit of the one-sided 99% confidence interval over the 99th percentile (UTL 99/99) of total residue concentrations in salmon derived from the pivotal study used for this assessment. The time-point at which the MRLs were set was based on the approach described at the 66th Meeting of the Committee.

The Committee recommended MRLs for emamectin B1a in salmon of 100 µg/kg in muscle and fillet, and extended these MRLs to trout.

The EDI is 11 µg/person per day, which represents 37% of the upper bound of the ADI.

Table 4.9. The estimated dietary intake of emamectin residues in salmon or trout tissues

Tissue	Median residue (µg/kg)	Standard Food Basket (kg)	MR:TR ratio	Estimated Daily Intake (µg)
Muscle	33	0.3	0.9	11
			EDI	11
			% of ADI	37%

NOTES: MR:TR ratio is the ratio of marker residue to total residues

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