Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice

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Abstract

Atlantic salmon (weight range 289–484 g) and rainbow trout (weight range 166–387 g), held in seawater, were fed medicated diets containing graded levels of emamectin benzoate for 7 days. Nominal dose rates were 0, 100, 250 and 500 μg kg⁻¹ body weight day⁻¹ (equivalent to 0, 2, 5 and 10 times recommended dose rate for treatment of sea lice). Fish were observed for a further 7 days to determine the effects of treatment. Calculated actual dose rates, compensated for feed concentrations determined by chemical analysis, feed intake and weight gain during the course of the study, were 0, 70, 173 and 356 μg kg⁻¹ body weight day⁻¹ for Atlantic salmon, and 0, 88, 218 and 413 μg kg⁻¹ body weight day⁻¹ for rainbow trout. No mortality was observed, which could be related to treatment and unequivocal signs of toxicity were recorded only at the highest dose used in these studies. Signs of emamectin benzoate toxicity in both species included lethargy, dark coloration and inappetance. Atlantic salmon also showed loss of coordination. Fish in the high-dose groups exhibiting signs of toxicity showed no evidence of recovery during the 7-day post-treatment period. No pathognomonic signs of emamectin benzoate toxicity were identified...
1. Introduction

Ivermectin (22,23-dihydroavermectin B₁) has been available worldwide for many years as a parasiticide for cattle, sheep, pigs, horses and dogs (Sutherland, 1990). A review by Pulliam and Preston (1989) of the toxicity of the compound in these species indicated that the recommended dose rates of up to 300 µg kg⁻¹ body weight had wide therapeutic indices. However, although approved at a dose of only 6 µg kg⁻¹ body weight for the prevention of heartworm disease, it was apparent that extra-label use of 100 µg kg⁻¹ body weight and greater resulted in severe toxicity in some individuals of the Collie breed of dog, associated with higher than expected concentrations of the drug in the central nervous system. In general, the acute toxic syndrome in mammals was characterised by depression, mydriasis, ataxia, coma and death. No specific gross or histologic changes were attributed to ivermectin. Recently, it has been used in feed for the control of sea lice in farmed Atlantic salmon in the UK when prescribed under the 'cascade' principle (Anonymous, 1998), subject to consent from the relevant Environmental Protection Authority. In general, a dose of 25 µg kg⁻¹ body weight day⁻¹, twice weekly for a period of 4 weeks, has been employed (Rae, 1996). Palmer et al. (1987) found increased mortality and listless behaviour, but no significant histopathological changes, among Atlantic salmon treated with a single oral dose of ivermectin at 400 µg kg⁻¹ body weight. O’Halloran et al. (1992) recorded increased mortality, and increased numbers of fish displaying signs of lethargy in Atlantic salmon smolts, held in freshwater, treated with a single dose of 200 µg kg⁻¹ body weight. Johnson et al. (1993) investigated the toxicity and pathological effects of orally administered ivermectin in laboratory studies with Atlantic salmon, coho salmon (Oncorhynchus kisutch), chinook salmon (O. tshawytscha), and steelhead trout (O. mykiss). In the Atlantic, coho and chinook salmon, no specific histopathological changes were associated with ivermectin toxicity, but gross changes were observed. Atlantic salmon became dark and inappetant, their eyes rolled ventrally so lenses were no longer visible, and intestinal congestion was recorded. Chinook and coho salmon became dark, with no changes in their eyes. The results of the study using steelhead trout were difficult to interpret due to a Vibrio infection. Toovey et al. (1999) claimed that ivermectin, formulated as Ivomec® Injectable Solution (Merial Animal Health, London, UK), caused a significant dose-dependent depression of oxygen consumption by isolated, rainbow trout gill tissue. However, as only one component of the injectable vehicle, propylene glycol, was examined in control preparations, and then only at concentrations much lower than in the ivermectin exposures, their conclusions cannot be regarded as valid.

A novel avermectin, emamectin benzoate (the benzoate salt of 4'-deoxy-4'-epi-(methylamino) avermectin B₁) has been developed as a treatment for sea lice of farmed salmonids in the UK, Norway, Chile and Canada. The compound is incorporated in feed...
as a 0.2% premix (Slice® Aquaculture Premix, Schering-Plough Animal Health) and a
dose of 50 µg kg⁻¹ body weight day⁻¹, for 7 days, is effective against immature and
adult stages of sea lice (Stone et al., 1999).

Emamectin benzoate is a semi-synthetic avermectin insecticide, originally developed
for pest control in edible plant crops (Lasota and Dybas, 1991). Avermectins are
considered to act by binding to glutamate-gated chloride channels, increasing neurone
permeability to chloride ions at invertebrate inhibitory synapses, resulting in paralysis
and death (Arena, 1994; Arena et al., 1995; Vassilatis et al., 1997). Toxicity data
generated in support of plant applications have shown that rodent LD₅₀ values ranged
from 22 to 120 mg kg⁻¹, with the CF-1 strain of mouse being most sensitive (Wislocki,
personal communication). Approximately 25% of the population of this strain of mouse
is deficient in P-glycoprotein, a component of the blood–brain barrier, which has been
shown to be important in preventing accumulation of avermectins in the brain (Schinkel
et al., 1994; Lankas et al., 1997).

The toxicity of emamectin benzoate to fish has been evaluated over a 96-h exposure
to compound in water. While the three freshwater species, rainbow trout (O. mykiss),
bluegill sunfish (Lepomis macrochirus) and fathead minnow (Pimephales promelas)
had LC₅₀ values ranging from 174 to 194 µg l⁻¹, a marine species, the sheepshead
minnow (Cyprinodon variegatus), was around eightfold less sensitive, with an LC₅₀ of
1340 µg l⁻¹ (Wislocki, personal communication). In comparison, 96 h LC₅₀ values for
rainbow trout and bluegill sunfish exposed to ivermectin were 3.0 and 4.8 µg l⁻¹,
respectively (Halley et al., 1989). These data suggest that, when exposed to compound
in water, fish are much less tolerant of ivermectin, than they are of emamectin benzoate.

In this report, we present details of two studies, conducted to determine the tolerance
of farmed Atlantic salmon, Salmo salar L., and rainbow trout, O. mykiss (Walbaum), to
emamectin benzoate incorporated into feed at 2, 5 and 10 times the recommended dose
rate. These studies were intended to provide an indication of the margin of safety for this
compound when used to control sea lice infestations of farmed salmonids.

2. Materials and methods

Both studies were conducted in accordance with Good Laboratory Practice using
facilities at the University of Stirling, Marine Environmental Research Laboratory,
Machrihanish, Argyll, UK. The first, using Atlantic salmon, was conducted in the
autumn of 1994, and the second, using rainbow trout, was conducted in the autumn of
1997. Both stocks of fish originated from University of Stirling, Howietoun Fishery,
Stirling, UK.

Eight 1.4-m diameter glass-reinforced plastic tanks (800 l volume) were used for each
study. Tanks were supplied with seawater at a flow rate exceeding 1 l min⁻¹ kg⁻¹ body
weight and additional aeration was supplied to all tanks throughout both trials. Tank
outlets were protected with fine (1.5-mm diameter mesh) screens to trap uneaten feed.
Tanks were cleaned, disinfected and rinsed before stocking with fish. Fish densities were
in the range 5–30 kg m⁻³. No medication other than the test compound was adminis-
tered during the course of the studies. Fish were fed by hand. During acclimatisation and
after treatment, both stocks were fed appropriate commercial diets at a rate of 0.7% body weight day$^{-1}$. Tank outlets were flushed each day, and tanks were brushed and siphoned when necessary to remove uneaten feed and faeces. During treatment and post-treatment, tanks were siphoned clean when uneaten feed was collected.

Water temperature, salinity and dissolved oxygen concentrations were measured once each day. In the Atlantic salmon study, water temperature fell from approximately 14°C to 10°C and salinity ranged from 32 to 34 ppt. In the rainbow trout study, water temperature was in the range 11°C–13°C and salinity was greater than 28 ppt. Dissolved oxygen concentrations were above 8 mg l$^{-1}$ throughout both studies.

2.1. Medication of feed

A total of 160 Atlantic salmon (weight range 289–484 g) were allocated randomly, 20 fish/tank, to the eight experimental tanks. Fish were allowed to acclimatise for 17 days before the start of treatment. Emamectin benzoate (technical grade), with a purity of 94.6%, was supplied by Schering-Plough Animal Health, Union, NJ, USA. Medicated diets were prepared to give nominal doses of 0, 100, 250 and 500 μg emamectin benzoate kg$^{-1}$ body weight day$^{-1}$ when fed a ration equivalent to 0.5% body weight day$^{-1}$. A stock solution was prepared by dissolving 848.0 mg emamectin benzoate in 10.00 ml propylene glycol (Sigma, Poole, UK) at room temperature. Medicated diets were prepared by combining measured aliquots of this solution and/or propylene glycol in cod liver oil (Seven Seas Health Care, Marfleet, UK). Commercial salmon feed pellets (BOCM Pauls, Renfrew, UK) were coated with the oil mixture (20 ml oil mixture/1604 g feed) by placing the feed and oil mixture in a plastic bag and agitating and turning the sealed bag. Pellets were then left overnight to allow the oil to be absorbed.

A total of 128 rainbow trout (weight range 166–387 g) were allocated randomly, 16 fish/tank, to the eight experimental tanks. Fish were allowed to acclimatise for 15 days before the start of treatment. A formulated premix (Trademark Slice® Aquaculture Premix, Schering-Plough Animal Health, Union, NJ, USA) containing 0.2% emamectin benzoate was supplied. Medicated diets were prepared to give nominal doses of 0, 100, 250 and 500 μg emamectin benzoate kg$^{-1}$ body weight day$^{-1}$ when fed at 0.5% body weight day$^{-1}$. Medicated diets were prepared by slowly adding the formulated premix to commercial trout feed pellets (BOCM Pauls, Renfrew, UK) during mixing, and then coating the medicated feed pellets with cod liver oil (15 ml kg$^{-1}$ feed). Pellets were then left overnight to allow the oil to be absorbed.

In both studies, prior to use, diets were stored in a secure, cool, dry location in sealed, labelled, containers. Samples of each diet were collected before and after treatment for emamectin benzoate determination using high-performance liquid chromatography (Farer et al., 1999).

2.2. Treatment protocol

In both studies, tanks were blocked to give two replicates and treatments were assigned randomly to tanks within replicate blocks. The experimental diets were
administered for a 7-day period (days 0–6) at a rate of 0.5% body weight day$^{-1}$. The
daily ration was fed by hand in one or two meals and, following each meal, uneaten
pellets were collected by siphon and counted. The weight of uneaten feed was estimated
from the number of pellets recovered and the average individual pellet weight. Prelimi-
nary tests using empty tanks gave recovery efficiencies of 100%. After treatment, from
days 7 to 12, fish were offered the same commercial diet used during acclimatization.
Feed intake estimations continued from days 7 to 12 to provide an indication of the
effects of treatment on appetite.

2.3. Observations

Fish were observed routinely during the two studies and any deviation from normal
behaviour and appearance was recorded. Any fish which died was subjected to gross
examination and tissue samples were collected for histological examination. All surviv-
ing fish were examined on day 13 by one of the authors (H.D.M.R.) who was unaware
of the allocation of treatments to tanks. Behaviour and appearance of fish in each tank
were observed and recorded. All remaining fish were then sacrificed, weighed and
measured and subjected to gross necropsy. Tissues for histological examination were
collected from any abnormal fish plus five fish selected at random from each tank
population. Tissue samples collected for histological examination included brain, gill,
liver, kidney and spleen, fixed in 4% phosphate-buffered formaldehyde. Fixed tissues
were embedded in paraffin wax and processed to haematoxylin- and eosin-stained
sections for examination.

3. Results

3.1. Dose delivered

In the salmon study, measured emamectin benzoate concentrations in medicated feed
samples were between 74.3% and 76.2% of those expected. Medicated feed intake,
determined by counting uneaten pellets, was greater than 99% in all tanks during the
course of the 7-day treatment period. The mean weight of the experimental fish
increased from 381.8 g on day −17 to 429.6 g on day 13, equivalent to a specific
growth rate (SGR) of 0.393% body weight day$^{-1}$. The ration used during the course of
the experiment was determined according to the mean weight of fish on day −17.
Assuming exponential growth over the study period, the mean weight of fish on day 0
was calculated to be 408.2 g and expected dose rates corrected for fish weight on day 0
were 93.5% of nominal. As a result, the emamectin benzoate dose levels, corrected for
measured concentrations in feed, percentage feed consumption and weight gain, were
less than the nominal dose rates. Minimum dose rates calculated for the salmon study
were 0, 70, 173 and 356 μg kg$^{-1}$ body weight day$^{-1}$ as shown in Table 1.

In the trout study, measured emamectin benzoate concentrations in medicated feed
samples were 85.2%–85.8% of those expected. Medicated feed intake during the 7-day
treatment period was greater than 99% in all treatment groups, except for the high-dose
Table 1
Expected and measured dietary emamectin benzoate concentrations, feed intake during treatment period and expected and calculated minimum dose rates, during the Atlantic salmon tolerance study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Low dose</th>
<th>Medium dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected dietary emamectin benzoate concentration (mg kg(^{-1}))</td>
<td>0</td>
<td>21.2</td>
<td>52.9</td>
<td>105.7</td>
</tr>
<tr>
<td>Measured dietary emamectin benzoate concentration (mg kg(^{-1}))</td>
<td>0</td>
<td>16.0</td>
<td>39.3</td>
<td>80.6</td>
</tr>
<tr>
<td>Overall percentage of medicated feed consumed</td>
<td>99.6</td>
<td>99.5</td>
<td>99.7</td>
<td>99.8</td>
</tr>
<tr>
<td>Nominal dose ((\mu g\ kg(^{-1}) body weight day(^{-1}))</td>
<td>0</td>
<td>100</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Calculated dose ((\mu g\ kg\ body weight day(^{-1}))</td>
<td>0</td>
<td>70</td>
<td>173</td>
<td>356</td>
</tr>
</tbody>
</table>

group where medicated feed consumption was 93.7% of the total ration offered. The mean weight of the experimental fish increased from 272.8 g on day -16 to 299.9 g on day 13, equivalent to an SGR of 0.327% body weight day\(^{-1}\). The ration used during the course of the experiment was determined according to the mean weight of fish on day 0, assuming an SGR of 0.5% body weight day\(^{-1}\) from the start of acclimatisation. Using the calculated SGR of 0.327, the mean calculated weight of fish on day 0 was 287.4 g and expected dose rates corrected for fish weight on day 0 were 102.8% nominal. Dose levels of emamectin benzoate, corrected for measured concentrations in feed, percentage feed consumption and weight gain, were again less than the nominal dose rates. Minimum dose rates in the trout study were calculated to be 0, 88, 218 and 413 \(\mu g\ kg\(^{-1}\) body weight day\(^{-1}\) as shown in Table 2.

3.2. Effects of emamectin benzoate toxicity

During the salmon study, one fish from the high-dose treatment group died on day 8, 2 days after the completion of treatment. No other mortalities occurred either during or after treatment. Pathological findings included focal necrosis, ceroid accumulation in the spleen and melanin accumulation in the kidney. These were consistent with prolonged anorexia and there was no evidence of any other clinical condition.

At the start of treatment, most fish were pale in colour and swam with a steady tail beat and with pectoral fins pulled close to the body. Most fish swam in groups in mid-water, close to the wall of the tank and facing into the current, although individuals frequently left the groups and darted towards the centre of the tank. When feeding, fish

Table 2
Expected and measured dietary emamectin benzoate concentrations, feed intake during treatment period and expected and calculated minimum dose rates during the rainbow trout tolerance study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Low dose</th>
<th>Medium dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected dietary emamectin benzoate concentration (mg kg(^{-1}))</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Measured dietary emamectin benzoate concentration (mg kg(^{-1}))</td>
<td>0</td>
<td>17.1</td>
<td>42.6</td>
<td>85.8</td>
</tr>
<tr>
<td>Overall percentage of medicated feed consumed</td>
<td>99.8</td>
<td>99.7</td>
<td>99.4</td>
<td>93.7</td>
</tr>
<tr>
<td>Nominal dose ((\mu g\ kg(^{-1}) body weight day(^{-1}))</td>
<td>0</td>
<td>100</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Calculated dose ((\mu g\ kg\ body weight day(^{-1}))</td>
<td>0</td>
<td>88</td>
<td>218</td>
<td>413</td>
</tr>
</tbody>
</table>
responded vigorously, taking feed pellets just below the water surface directly under the feeding hatch. On day 5 of treatment, one lethargic fish was observed in the high-dose group. On day 6, several fish in both replicate high-dose tanks were inactive, and the majority were stationed on the bottom of the tank rather than in mid-water as described above. One fish in each of the high-dose tanks was observed to roll onto its side at intervals, apparently unable to maintain an upright position. On day 7, a fish in one of the high-dose tanks appeared moribund but swam off when disturbed. This fish was found dead on day 8. A second fish in this tank exhibited similar behaviour from day 8, and a third in the same tank was observed lying on its side from day 10. On day 10, the majority of fish in the high-dose treatment tanks was observed to be darker in colour than fish in the other tanks. Feeding response in the high-dose tanks was poor from day 8, with fish no longer rising to take feed pellets just below the water surface. White faecal casts, indicative of lack of feeding, were observed in these tanks from day 10. Feed intake, determined by recovery of uneaten pellets, remained high in all tanks until day 9 when the number of feed pellets recovered from the high-dose tanks increased sharply. Total feed intake during the post-treatment period, days 7–12, was greater than 97% of feed offered in all tanks except the two high-dose replicates, where feed intake was 39.9% and 48.5% of total ration, respectively. Fish in the high-dose tanks showed no evidence of recovery of normal behaviour or appearance before the end of the study.

On day 13, detailed examination of fish by an observer who was unaware of the allocation of treatments to tanks confirmed that all fish in high-dose replicate 1 were dark in colour, and that two of these fish showed abnormal behaviour, appearing to exhibit lack of co-ordination. White intestinal casts, indicating lack of feeding, were visible on the floor of the tank. In high-dose replicate 2, the majority of fish were lethargic and dark in colour (4/20 showed normal coloration). Some fish were at the water surface and one fish showed several small, superficial, flank lesions. One fish in medium-dose replicate 1 appeared slightly lethargic and one fish in medium-dose replicate 2 was slightly lethargic and also darker in appearance than other fish in the tank. No abnormalities were detected in fish in low-dose replicate 1. One fish in low-dose tank replicate 2 appeared slightly lethargic and was dark in appearance and another fish exhibited exophthalmus. All other stocks showed no significant clinical findings. Two apparently abnormal fish (fish numbers 1 and 4) were examined from high-dose replicate 1. Fish 1 showed a ventral skin lesion and erosion of the pectoral fins, nose and mandibles. Fish 4 showed unilateral corneal oedema, a ventral skin lesion and erosion of the nose and mandibles. White casts were recorded in the intestines of both fish. Tissues from these fish were examined histologically. Fish 1 showed an anterior subcapsular cataract. There were no significant findings in fish 4.

Gross examinations were conducted on all remaining fish on day 13. A high prevalence of pectoral fin and mandible erosion and a lower prevalence of nose erosion and flank lesions were recorded in all tanks. Corneal oedema and cataract were recorded in 15% of the control fish, 48% of low-dose fish, 48% of medium dose fish and 28% of high-dose fish. No feed was present in the intestine of 74% of fish from high-dose treatment. Feed was present in the gut of all but one or two fish in each of the other tanks. Ventral skin lesions were recorded in 14 fish (35%) from the high-dose group and one fish from the medium dose group, but were not recorded from low-dose or control.
A variety of histological lesions was recorded, including focal liver necrosis, cardiomyopathy, melanin accumulation in the kidney and spleen, and ceroid accumulation in the spleen and liver, but none showed any consistent relationship with either treatment or dose.

In the trout study, only one fish died during the treatment period and this was not considered to result from treatment. This fish, from low-dose replicate 2, died on day 1. No feed was observed in its gut. Histopathology showed scattered melanin granules in the spleen, high melanin levels in the kidney and a slight hypertrophy of the gill epithelia. High melanin levels in the kidney indicated that the fish had been anorexic for some time and the scattered melanin in the spleen suggested toxic effects as seen with bacterial toxins. No other mortalities occurred either during or after treatment.

Prior to the start of treatment, most fish swam with a steady tail beat, close to one another, close to the bottom of the tank and facing into the water current, swimming to maintain position within the tank, though individuals frequently left the groups and darted around the tank. The colour of the fish varied from pale through dark green/grey, but single dark fish were observed in control tank replicate 1 and low-dose replicate 2. Feeding response prior to the start of treatment was fair or good in all tanks, with fish actively seeking feed pellets above the floor of the tank, or rising to take feed pellets just below the water surface. During the treatment and post-treatment periods, feeding response in most tanks was generally fair or good, but from day 5, feeding response in both high-dose tanks was described as fair or poor, with fish not rising to the water surface, but either actively seeking feed only deeper in the tank or not actively seeking feed at all. On day 7, following the last day of treatment, four dark fish (25%) were observed in high-dose replicate 1, and three (19%) in high-dose replicate 2. Total feed intake during the post-treatment period, days 7–12, was greater than 96% of feed offered in all tanks except in the high-dose tanks where feed intake was 55.0% of total ration offered. In these high-dose tanks, no evidence of recovery of appetite was noted before fish were sacrificed at the end of the post-treatment period.

On day 13 (end of study), observations, made without knowing the identity of the treatments, confirmed signs of toxicosis in fish in the two high-dose tanks and in medium-dose replicate 2. Observations of behaviour and appearance made prior to capture of fish for sampling confirmed that some fish in these tanks were lethargic and exhibited increased melanization of the skin. No other abnormalities in appearance or behaviour were observed. Gross pathology was predominantly that associated with physical trauma and was not specific to tanks with dark and lethargic fish. A high proportion of fish in both high-dose tanks and medium-dose replicate 2 was anorexic. Although a number of fish in all tanks were maturing — and maturing fish often cease to feed — the high proportion of anorexic fish in these tanks was considered not simply to be a result of maturation. As in the salmon study, no specific histopathological findings were considered indicative of emamectin benzoate toxicosis.

Clinical examinations at the end of the trout study showed that some fish in all treatment groups had no feed in their gastrointestinal tract at the time of sampling. Fish were not fed on the day of slaughter, but feed offered the previous day would be expected to be present after 24 h. In the low-dose and control groups, the incidence of anorexia was 17% (5/30 fish) and 26% (8/31 fish), respectively. The highest incidence
of anorexia was recorded in the high-dose group where 50% (16/32) of the fish was found to be inappetant. In medium-dose replicate 1, 20% (3/15) of the fish was anorexic at slaughter, and feed intake was close to 100%, similar to findings in control and low-dose tanks. However, in medium-dose replicate 2, 56% (9/16) of the fish was found to be anorexic, yet post-treatment feed intake was greater than 96% of ration offered.

4. Discussion

Calculated dose rates were lower than nominal in both studies. In the salmon study, this was due to the combined effects of low analytical recoveries of emamectin benzoate from feed samples, and underestimated fish weight during the treatment period. Compensation for the effects of fish growth was made in the trout study, but low analytical recoveries of dietary emamectin benzoate were again recorded. As a precaution, to avoid the risk of overestimating tolerance levels, pathological findings are related to the calculated minimum dose rates shown.

The cause of death of the single salmon which died on day 8 was not established with certainty and mortality was not consistently found in the high-dose groups. Consequently, we conclude that mortality was not a feature of emamectin toxicity over the dose range used, but may result at higher doses than those tested in the present study.

No pathognomonic signs of emamectin benzoate toxicity were identified during gross necropsy or histopathological examination. In the absence of indications of any other clinical condition, the dark coloration and abnormal behaviour (lethargy, incoordination) of fish in both studies treated at the high dose were regarded as indicative of a direct toxic effect of emamectin benzoate. This interpretation is supported by similar observations in Atlantic salmon treated with high levels of ivermectin (Palmer et al., 1987; H.D.M. Rodger, personal observations). Fish in the high-dose groups exhibiting signs of toxicity showed no evidence of recovery during the 7-day post-treatment period. Due to its low prevalence within the tank populations, the cause of the abnormal behaviour and appearance of individual fish in medium- and low-dose groups could not be established. Although these observations may be related to emamectin benzoate toxicity in individual fish, we conclude that they are not consistent findings at the low- and medium-dose levels tested. Individual variation in feed intake during the treatment period would result in exposure of voracious individuals in all tanks to higher levels of emamectin benzoate than intended. This may explain the observation of individual fish with signs of toxicosis in low- and medium-dose groups.

Fin, nose and mandible erosion and skin lesions observed on gross examination of apparently normal fish were considered to result from physical trauma and tank abrasion. The higher prevalence of ventral skin lesions among fish in the high-dose group was attributed to increased contact with the bottom of the tank and is consistent with the observation of lethargy in this group. Eye pathology (corneal oedema, cataract) recorded in the salmon study was also consistent with external physical trauma and was not considered to be due to any direct effect of treatment with emamectin benzoate. Although the prevalence of these lesions was higher in treated fish than in controls, the
conclusion that these resulted directly from treatment was rejected since there was significant tank-to-tank variation in the number of fish affected, eye pathology was frequently unilateral, and there was no apparent association between dose level and prevalence or severity. Subsequent examination of the stock of fish, from which the experimental animals were drawn, confirmed the presence of similar lesions in fish not used for the study.

Feed consumption measurements show that feed intake was reduced in the latter part of both studies, indicating that emamectin benzoate toxicity may cause anorexia. In the trout study, a proportion of fish in all groups was anorexic. Several factors unrelated to emamectin benzoate toxicity, including maturation, effects of seawater transfer, social interactions, and physical trauma, may account for this underlying level of anorexia.

In efficacy studies with Atlantic salmon in tanks, no adverse effects were recorded at doses of up to 100 \( \mu g \) kg\(^{-1}\) body weight day\(^{-1}\) for 7 days (Stone et al., 1999). The design of the present study does not permit a precise calculation of a therapeutic index (median LD\(_{50}\)/median ED\(_{50}\)), but does demonstrate that emamectin benzoate is tolerated by Atlantic salmon, held at 10\(^\circ\)C–14\(^\circ\)C, at dose rates of at least 173 \( \mu g \) kg\(^{-1}\) body weight day\(^{-1}\) (3.4 \( \times \) recommended dose) and by rainbow trout, held at 11\(^\circ\)C–13\(^\circ\)C, at dose rates of at least 218 \( \mu g \) kg\(^{-1}\) body weight day\(^{-1}\) (4.3 \( \times \) recommended dose). Signs of toxicity were identified at dose rates of 356 \( \mu g \) kg\(^{-1}\) body weight day\(^{-1}\) in salmon (7.1 \( \times \) recommended) and 413 \( \mu g \) kg\(^{-1}\) body weight day\(^{-1}\) in trout (8.3 \( \times \) recommended dose).

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References


Rae, G.H., 1996. Guidelines for the use of ivermectin premix for pigs to treat farmed salmon for sea lice. Scottish Salmon Growers Association Pamphlet.


