

Clinical efficacy of teflubenzuron (Calicide®) for the treatment of *Lepeophtheirus salmonis* infestations of farmed Atlantic salmon *Salmo salar* at low water temperatures

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ABSTRACT: The efficacy of teflubenzuron (Calicide®) for the treatment of farmed Atlantic salmon *Salmo salar* L. infested with sea lice *Lepeophtheirus salmonis* (Krøyer, 1838), was investigated at low water temperatures in 2 commercial salmon farms. Calicide®, coated on commercial feed pellets, was administered orally at 10 mg kg⁻¹ d⁻¹ for 7 consecutive days. Fish were randomly sampled and lice numbers recorded from both treated and control groups on 3 or 4 sampling occasions post-medication. Statistically significant reductions in the number of *L. salmonis* per fish were recorded. Maximum efficacy was observed toward chalimus and preadult stages of *L. salmonis*, and was achieved approximately 26 d post-medication. No adverse drug reactions or palatability problems were associated with the treatments.

KEY WORDS: Sea lice · *Lepeophtheirus salmonis* · Efficacy · Teflubenzuron

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INTRODUCTION

Sea lice (Copepoda: Caligidae) *Lepeophtheirus salmonis* (Krøyer, 1838) are a serious pathogen of farmed Atlantic salmon *Salmo salar* L., in Europe and North America (Wootten et al. 1982, Pike & Wadsworth 1999). *L. salmonis* has a direct life cycle, comprising 10 stages: 2 planktonic naupliar stages, 1 infective copepodid stage, 4 chalimus larval stages, 2 preadult stages, and 1 adult stage (Johnson & Albright 1991, Schram 1993). The chalimus stages are physically attached to the fish by a frontal filament (Bron et al. 1991), while the other stages are mobile.

Management of sea lice infestations is achieved through the use of good management practice (Grant & Treasurer 1993), cleaner fish (Costello 1993) and chemotherapeutants (Roth 2000). The principal chemo-

therapeutic agents currently used to control sea lice under commercial conditions in various countries include dichlorvos (Aquagard®, Novartis), azamethiphos (Salmosan®, Novartis), hydrogen peroxide (Paramove®, Solvay-Interox; Salartect®, Brenntag), cypermethrin (Excis®, Betamax®, Novartis) and deltamethrin (Alphamax®, Alparma), which are administered as bath treatments; and emamectin benzoate (Slice®, Schering Plough), diflubenzuron (Lepsidon®, EWOS) and teflubenzuron (Calicide®/Ektobann®, Nutreco Aquaculture), which are administered orally (Roth 2000). Reduced sensitivity to several of these products has been reported (Jones et al. 1992, Treasurer et al. 2000).

Teflubenzuron is an insect growth regulator of the acylurea group which interferes with the synthesis of chitin in insects (Clark & Jewess 1990, Ishaaya & Klein 1990, Ah-Sun 1991) and sea lice (Grøntvedt 1996, Branson et al. 2000). The mode of action of the compound led to its consideration as a possible candidate for the control of *Lepeophtheirus salmonis*. Registered

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as Calicide® in the UK (a medicinal premix containing 100% teflubenzuron) and Ektobann® in Norway (a medicated feed containing 2 kg teflubenzuron per tonne fish feed), teflubenzuron has been used as a treatment for sea lice infestations in Norway since 1996 and in the UK since 2000 (Branson et al. 2000, Ritchie unpubl. data). Branson et al. (2000) documented that teflubenzuron has been extensively tested as a treatment for *L. salmonis* and demonstrated that when administered orally at 10 mg kg⁻¹ fish d⁻¹ for 7 consecutive days teflubenzuron was highly effective in trials conducted at water temperatures ranging from 11 to 15°C. Branson et al. (2000), however, did not address the question of efficacy at low water temperatures, and consequently questions as to whether adequate tissue levels would be achieved at low water temperatures, and whether or not chitin production in the sea lice would be sufficient at such temperatures to be affected by the teflubenzuron, were not answered.

Teflubenzuron has been shown to achieve therapeutic levels in the skin and muscle of Atlantic salmon at temperatures of 10 and 6°C when given at a dose rate of 10 mg kg⁻¹ body wt d⁻¹ for 7 d (Hoff et al. 1997, Auger et al. 1999).

The aim of the present study was to evaluate the clinical efficacy of teflubenzuron towards infestations of *Lepeophtheirus salmonis* under commercial conditions at low water temperatures.

MATERIALS AND METHODS

Clinical trials were conducted in 1995 and 1996 at 2 commercial Atlantic salmon sea sites on the west coast of Norway, Hordaland County (Site 1) and More and Romsdal County (Site 2). In compliance with regulatory guidelines, both trials were conducted according to the 'Principles of Good Clinical Practice'. Descriptions of each site and the lice treatment histories are given in Table 1.

Trials were designed with a ratio of 1 untreated control cage to 4 or 5 treated cages. The position of the control cages was randomised. Details pertaining to the dates of treatment, sampling dates and water temperatures are summarised in Table 2. Sampling to determine levels of infection per fish in each cage at the start of the trials was carried out pre-treatment on both sites. Sampling was also carried out at 3 or 4 post-treatment sampling points. All cages were sampled at each sampling point, the sample size being 20 fish from the control cage, 10 from each treated cage. Each cage therefore acted as an experimental unit. A random sample of fish from each cage was ensured by the use of a sweep net to crowd the fish within the cages, followed by a dip net to collect the sample. Fish were killed and, using a hand lens and torch, all lice present on the fish were counted and identified by species and life cycle stage.

Table 1. *Salmo salar*. Site-specific details (cages, year-class, number, biomass and weight of fish) and sea lice *Lepeophtheirus salmonis* treatment histories for Trial Sites 1 and 2

Site	Cages (dimensions)	Year-class	No. of fish	Biomass (t)	Fish weight (kg)	Lice treatments
1	6 Polarcircles (60 m circumference)	1995	299 357	40.26	0.135 ± 0.009	None
2	8 Polarcircles (60 m circumference) ^a	1995	167 000	127.86	0.732 ± 0.308	1995: 1 (azamethiphos)

^aThree cages were empty; therefore 5 were used in the trial

Table 2. *Salmo salar*. Treatment dates, number of treated and control cages, sampling dates and mean water temperatures for Trial Sites 1 and 2. Sampling dates include study day in parentheses, Day 1 being the first day of medication

Site	Treatment dates	No. treated cages	No. control cages	Sampling date	Mean water temperature during treatment (range) (°C)
1	05–11 Dec 1995 (Days 1–7)	5	1	04 Dec 1995 (Day 1)	7.1 (5.6–8.3)
				12 Dec 1995 (Day 8)	
	20–27 Dec 1995 (Days 16–22) (control group)			19 Dec 1995 (Day 15)	
				03 Jan 1996 (Day 30)	
2	19–25 Feb 1996	4	1	16 Feb 1996 (Day 3)	5.4 (5.4)
				01 Mar 1996 (Day 12)	
				08 Mar 1996 (Day 19)	
				15 Mar 1996 (Day 26)	
				22 Mar 1996 (Day 33)	

Teflubenzuron (98.7% pure active ingredient confirmed by certificate of analysis) was supplied and surface coated onto a 3.0 mm basal diet at Site 1, 6.0 mm at Site 2 (Edel brand, Skretting AS, Averøy, Norway), at a rate of 2.0 g active ingredient kg⁻¹ feed by Skretting AS, followed by top-coating of the feed with fish oil. Chemical analysis of each medicated batch of feed confirmed inclusion levels of 2.04 ± 0.18 g kg⁻¹ (Site 1) and 2.03 ± 0.09 g kg⁻¹ (Site 2). Control feed was prepared with basal diet and fish oil only. Medicated feed was administered to the fish at 0.5% body wt d⁻¹ as the first feed of the day, resulting in a nominal dose of 10 mg kg⁻¹ fish biomass d⁻¹. Duration of medication was 7 d (Days 1 to 7). Care was taken with feed administration to maximise the likelihood of medicated feed being presented to all the fish within the pens. If the predetermined amount of medicated feed was not enough to meet the demands of the fish for the first feed of the day, unmedicated feed was supplemented. If the fish did not eat all the medicated feed during the first feed, the remaining medicated feed was given at the second feed, prior to unmedicated feed. Feed rates and feeding methods were identical for all cages in both trials. Mortalities were recorded 2 to 3 times per week.

Water temperatures were recorded at 2 m (Site 1) and 4 m (Site 2) depth, daily, for the treatment duration. As both sites were located close to the open ocean, salinity was assumed to remain stable throughout the trial period and was not measured.

Lice counts were summarised as copepodids, chalimus (Chalimus Stages I, II, III and IV), preadult (Preadult I and II), adult and total lice, and the arithmetic means were calculated. Data were subjected to statistical analysis by nested ANOVA with individual fish as factors, nested within pens, and pens nested within treatment groups. Tukey's honestly significant-difference (HSD) test was used to determine significant differences between treated and non-treated control groups.

Efficacy of treatment was defined as the percentage reduction in the mean total number of *Lepeophtheirus salmonis* per fish and the mean number of susceptible lice (copepodids, chalimus larvae and preadult) per fish in treated and control replicates from the pre-treatment intensities, according to the following equation:

$$\% \text{ efficacy } (t) = 100 - [(X_D / X_{D0}) \times 100]$$

where X_D is the mean number of lice per fish for replicates at D days post-treatment, and X_{D0} is the mean number of lice per fish for replicates at pre-treatment sampling.

RESULTS

Treatments

The total biomass treated, amount of medicated feed delivered, feeding rate and resultant dose rate achieved at both sites are shown in Table 3. The mean dose rate achieved was slightly higher than that intended at Site 1 (by 1.3 mg kg⁻¹ body wt d⁻¹) and slightly lower at Site 2 (by 1.9 mg kg⁻¹ body wt d⁻¹). Nominal feeding rate for both sites was 0.5% body wt d⁻¹ for 7 d. Actual mean feeding rates varied slightly from 0.56% (Site 1) to 0.41% (Site 2).

Clinical observations and mortalities

No abnormal or unusual behaviour was observed in any of the treated cages during treatments at either site. There was no evidence of reduced appetite or apparent palatability problems associated with the medicated feed in any of the cages.

The cumulative mortalities over the course of the trial and the percentage mortality of the initial population in each cage, at both sites, can be seen in Table 4. At Site 1, an outbreak of infectious pancreatic necrosis (IPN) was detected in Cage 2 following medication, accounting for the larger number of mortalities in this cage. Consequently, this pen was not used in post-treatment sampling. With the exception of this cage, cumulative mortalities were similar for treated and untreated control cages at both sites. There were no adverse drug reactions associated with the treatments.

Efficacy of treatments

There was a significant percentage reduction in the mean total number of *Lepeophtheirus salmonis* per fish and the mean number of susceptible lice (copepodids, chalimus larvae and preadult) per fish in the treated groups from pre-treatment to all post-treatment sampling points ($p < 0.01$, ANOVA and Tukey's HSD), in both trials (Table 5).

Table 3. *Salmo salar*. Treatment details for Trial Sites 1 and 2

	Site 1	Site 2
Treated biomass (kg)	33 993	100 860
Medicated feed per day (kg)	192	410
Feeding rate (% body wt d ⁻¹)	0.56	0.41
Dose received (mg kg ⁻¹ body wt d ⁻¹)	11.3	8.1

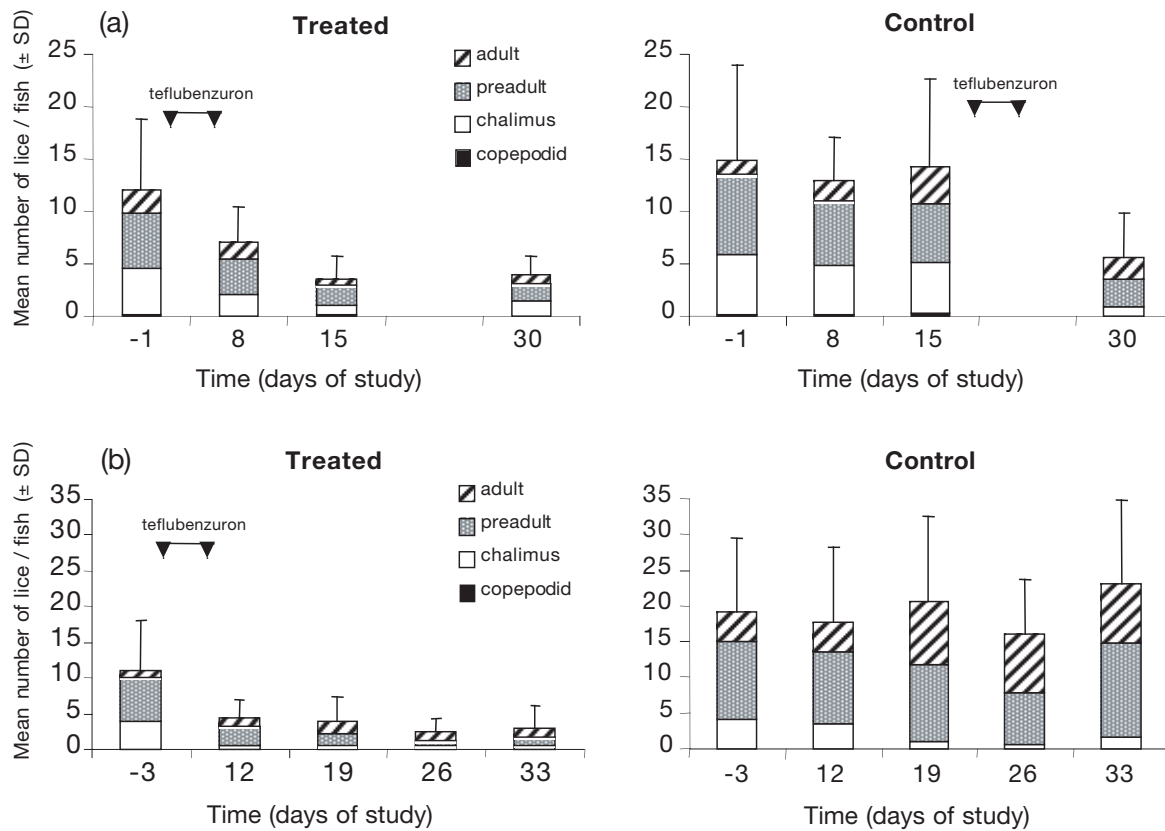


Fig. 1. *Lepeophtheirus salmonis*. Mean number of copepodids, chalimus, preadults and adults on Atlantic salmon *Salmo salar* treated with teflubenzuron (Days 1 to 7) at (a) Site 1 and (b) Site 2. Sample size = 10 fish per treated cage and 20 fish per control cage

Maximum efficacy towards the mean total number of lice per fish reached 69.4 and 77.5%, at Sites 1 and 2 respectively. Maximum efficacy towards the mean number of susceptible lice per fish reached 69.7 and 88.0%, at Sites 1 and 2 respectively.

At Site 1, the mean number of lice per fish was significantly lower in the treated group than in the control group at the first and second post-treatment sampling points (ANOVA, Tukey's HSD, $p < 0.001$) (Fig. 1a). The greatest reductions were seen in the mean number of

chalimus and preadult lice per fish. Although the mean number of adult lice was reduced to a lesser extent, this reduction was significant at the second and third post-treatment sampling points ($p < 0.01$). In the control group, the mean number of lice per fish remained relatively constant from pre-treatment until the second post-treatment sample, after which this group was treated with teflubenzuron on Day 16 and there was then a significant reduction in the mean number of lice per fish ($p < 0.001$).

Table 4. *Salmo salar*. Cumulative mortalities (N) and percentage mortality of the initial population in each cage at Site 1 (Days 1 to 27) and Site 2 (Days 1 to 14). Fish sacrificed for sampling not included

Cage	Site 1		Cage	Site 2	
	Cumulative mortality	% mortality		Cumulative mortality	% mortality
1	87	0.17	1	63	0.29
2	569	0.87	2 (control)	74	0.25
3	69	0.15	3	126	0.29
4	59	0.13	4	40	0.07
5 (control)	44	0.10	5	58	0.32
6	29	0.06			

At Site 2, the mean number of lice per fish was significantly lower in the treated group than in the control group at all post-treatment sampling points ($p < 0.001$; Fig. 1b). The greatest reduction was in the mean number of chalimus and preadult lice per fish. There was no reduction in the mean number of adults per fish in the treated group over the trial period ($p = 0.23$). The mean number of lice per fish in the control group remained relatively constant over the trial period; however, there was a rise in the number of adults present over the trial period ($p < 0.05$) as the lice naturally completed their life cycles.

DISCUSSION

Because fish are poikilotherms, the question arose as to whether there would be sufficient uptake of teflubenzuron from the gut at low water temperatures to achieve therapeutic levels in the skin and muscle of treated fish to control lice. In addition, because sea lice development rates slow significantly as temperatures decline (Johnson & Albright 1991), there was a possibility that there would be insufficient chitin deposition over the course of the treatment to delibitate the lice, thus making the treatment ineffective.

These trials showed the efficacy of teflubenzuron as a treatment for natural infestations of *Lepeophtheirus salmonis* down to water temperatures of 5.4°C. The numbers of susceptible *L. salmonis* per fish were reduced by up to 88.0%.

At Site 2, the mean total number of lice in the control cages remained approximately the same over the course of the trial, with populations developing naturally, adult numbers increasing, and chalimus decreasing in number as they developed. This is the pattern to be expected where there is no copepodid settlement. In the treated cages, adult numbers remained relatively constant, whereas the numbers of other stages declined, as would be expected. Numbers of developing stages had stabilised at the Day 26 count (numbers

at the Day 26 and Day 33 counts were approximately the same), probably indicating that the maximum efficacy at this temperature (5.4°C) was achieved on Day 26, or between Day 19 and Day 26.

At Site 1 there was a similar picture in the control cages to that at Site 2. In the treated cages, in addition to the decline in developing stages, there was also a significant decline in adults. The reason for this was not clear, but is likely to have been related to an environmental factor, for example the decrease in water temperature. It has been demonstrated that teflubenzuron does not affect adult *Lepeophtheirus salmonis* (Branson et al. 2000), as would be expected given the fact that teflubenzuron inhibits the biochemical synthesis of chitin, and therefore disrupts moulting, a process not occurring in adults. Because teflubenzuron is not indicated for the adult stages of *L. salmonis*, as stated by Branson et al. (2000), it was more appropriate to calculate efficacy in relation to susceptible stages (copepodids, chalimus and preadults) of lice.

From the example of Site 2, the lice numbers would have been expected to fall further to the third post-treatment sample point at Site 1, but this did not occur, and intensities were similar at both the second and third post-treatment sample points. Branson et al. (2000) found maximum efficacy of teflubenzuron toward *Lepeophtheirus salmonis* at 7 d post-medication, at water temperatures ranging from 11 to 15°C. The data from Site 2 suggests that maximum efficacy at 5.4°C was reached around 26 d post-treatment. As a consequence of this, because the average water temperature at Site 1 was 7.1°C, it seems likely that the point of maximum efficacy at Site 1 would have occurred before Day 26. Consequently, lice numbers may have fallen below the levels seen on Day 15, but risen again to the levels on Day 30 because of copepodid recruitment which occurred during this trial. Therefore, the true maximum efficacy in this trial is likely to have been higher than that calculated, but was not measured because of the failure to carry out post-treatment sampling at the appropriate time.

Table 5. *Salmo salar*. Percentage efficacy of teflubenzuron. Comparison of mean total number of *Lepeophtheirus salmonis* per fish (Efficacy, total) and mean number of susceptible lice (copepodids, chalimus larvae and preadult) per fish (Efficacy, susc.) in treated and control groups with pre-treatment lice levels, following treatment at Sites 1 and 2

Day	Site 1				Day	Site 2			
	Efficacy (total)		Efficacy (susc.)			Efficacy (total)		Efficacy (susc.)	
	Control	Treated	Control	Treated		Control	Treated	Control	Treated
8	12.7	40.5	18.5	44.4	12	2.2	59.5	10.5	67.0
15	4.0	67.8	20.0	67.7	19	-13.8	64.9	22.5	78.0
30	62.4 ^a	69.4	73.3 ^a	69.7	26	11.6	77.5	48.7	88.0
					33	-26	73.9	-2.6	84.0

^aControl group treated from Days 16 to 22

Teflubenzuron is clearly effective at low water temperatures, but the effect is delayed compared to that observed at higher temperatures (Branson et al. 2000). This is likely to be due to the longer period between moults of all stages of the sea lice, but particularly preadults, which have a long intermoult (Johnson & Albright 1991).

Copepodid recruitment was observed at Site 1. Although copepodids attaching during the early stage of the treatment will be killed, at some point attaching copepodids will survive, so lice numbers will not fall as low as they would without the presence of this recruitment. This recruitment would have arisen from adults within the medicated cages and also from the control cage. For maximum efficacy, therefore, it is important that treatment is carried out before the appearance of significant levels of adult lice. At Site 2 there was no recruitment. It is known that the reproductive output of *Lepeophtheirus salmonis* decreases at low water temperatures (Ritchie et al. 1993), which probably accounts for the absence of recruitment at this site.

Efficacy was achieved at Site 2 even though the dose rate used was lower than that intended (an average of 8.1 instead of 10 mg kg⁻¹ body wt d⁻¹). This was due to the fishes' appetite being lower than the anticipated 0.5% body wt d⁻¹, presumably because of the low water temperatures. Higher dose rates were used at Site 1 (11.3 mg kg⁻¹ body wt d⁻¹) due to overfeeding with medicated feed. Any feed given to the fish in excess of the planned 0.5% body wt d⁻¹ should have been unmedicated, but in fact medicated feed was used, with the resulting slight overdosage.

IPN occurred in one of the cages at Site 1 (Cage 2), and some losses resulted. In order not to cause these fish any undue stress that might have resulted in further losses, this cage was excluded from the post-treatment sampling. It is unlikely that this event affected the results of the trials.

In conclusion, these trials demonstrated that teflubenzuron can be an effective treatment for infestations of the developing stages of the sea lice *Lepeophtheirus salmonis* on Atlantic salmon at temperatures as low as 5.4°C when applied at a dose rate of 10 mg teflubenzuron kg⁻¹ body wt d⁻¹ for 7 d. Reduction in lice numbers was seen up to 26 d post-medication.

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