SCOTTISH ENVIRONMENT PROTECTION AGENCY

POLICY NO 29

Fish Farming Advisory Group

Calicide (Teflubenzuron) - Authorisation for use as an in-feed sea lice treatment in marine cage salmon farms. Risk Assessment, EQS and Recommendations

(As agreed at the Board Meeting held on 7th July, 1998 and subsequently updated in July, 1999)

Version 1.1
July 1999

1. EXECUTIVE SUMMARY

- The Scottish salmon farming industry is anticipating the release onto the market of "**Calicide"** which is a new in-feed sea lice treatment. It is likely that applications for consent to use Calicide at a number of marine cage fish farm sites will be sent in to SEPA during the summer.

- Field trials have shown limited but acceptable environmental risks from the use of Calicide.

- Calicide should be authorised for use for an initial 2 year period by the normal SEPA site-specific consent process.

- SEPA's Fish Farming Advisory Group (FFAG) will review these standards and their application in the light of any new scientific evidence such as from post-authorisation monitoring, and developments with predictive sediment impact models.

- FFAG will advise licensing teams on post-authorisation monitoring requirements.

- Revision 1.1 of Policy 29 takes account of additional information supplied in March 1999 from long-term monitoring of the field trial at Loch Eil and also the outcome of peer review by the Water Research Centre (WRc) on the derivation of SEPA standards for teflubenzuron.

2. BACKGROUND

2.1 **Calicide (active ingredient *Teflubenzuron*)** is a new in-feed sea lice treatment chemical for use by the marine salmon farming industry. Calicide has undergone extensive testing and field trialing since 1995 by the world's largest fish feed manufacturer, **Nutreco ARC Ltd**.

Nutreco has supplied SEPA's Fish Farming Advisory Group (FFAG) with a detailed dossier of ecotoxicological information (Anon., 1997 and Trouw, 1999), expert reports and reviews of the effects of Calicide (Jenkins 1995 and Baird et al. 1997, Baird 1998). Studies have been carried out in Scotland, Norway, Canada and Chile as sponsored and co-ordinated by Nutreco Aquaculture Research Centre (ARC) in Norway.

To date, Nutreco has costed the development of Calicide at around £3.4 million, of which some £900,000 has been for ecotoxicological studies.

Final reports are now available on field trials which were conducted in Scotland (McHenery and Ritchie, 1998 and Telfer, 1998) with additional long-term monitoring at one site from 1997 through to end 1998 (Trouw,1999). These studies were commissioned to meet the needs of both SEPA and the Veterinary Medicines Directorate (VMD) in order to supply validated field information on the dispersion, fate and field ecotoxicity of Calicide.

The required information is now complete and Nutreco wish to progress to marketing Calicide in Scotland.

The product will be **marketed in the UK by** Nutreco's subsidiary company, **Trouw UK Ltd**. There have been full, continuous discussions with SEPA's FFAG of study protocols and developments at each stage of the authorisation process.

Calicide now has a Marketing Authorisation (MA) from the VMD which was finally approved by their Veterinary Products Committee in July 1999. SEPA require fish farmers to ensure that appropriate medicinal authorisations are in place (Medicines Act and subordinate regulations).

There is presently an EU maximum residue limit (MRL) issued for teflubenzuron in salmon flesh of 500 ug/kg (ppb) which requires a 45 degree day minimum withdrawal period. The MRL is valid for an initial period of 2 years. Nutreco intend to inform users of Calicide to use a 100 degree day, or a fixed 7 day withdrawal period for all sea water temperatures, which will ensure that any residual levels of teflubenzuron in salmon flesh are well within the required MRL.

2.2 International issues

In other countries, the situation for Calicide use and authorisation is as follows:

- **Norway** - widespread use under General Exemption approval system since Sept 1997. To date over 300 treatments and around 750 tonnes medicated feed per annum. General Exemption extended from 1 May 1998 for a further 2 years. MA applied for in Sept 1997.
- **Canada** - MA application submitted Dec 1997 and result anticipated July 1998. Currently available for use under Emergency Drug Order (EDR) procedure on a farm application basis, first EDR granted Jan 1998.
- **Ireland** - MA application submitted Sept 1997, approval expected July 1998.
- **Chile** - current approval to use 1000 tonnes medicated feed as interim to amending registration procedures. MA will be registered once registration procedures are re-issued.

2.3 Teflubenzuron

The active ingredient in Calicide is teflubenzuron. Calicide (trade name for the medicated feed product) is prepared by coating commercial fish feed pellets with teflubenzuron as a powder to a level of 2g teflubenzuron/kg feed (0.2%w/w).

Teflubenzuron is a chitinase inhibitor and exerts toxicity by inhibiting the formation of chitin which is the predominant component of the exoskeleton of insects and crustacea. It therefore exerts an effect at a moulting stage in the life cycle of exposed organisms.

Teflubenzuron has a moderate octanol:water partition coefficient and relatively low water solubility which means that in the environment it tends to remain largely bound to sediment and organic material (Jenkins 1995, Baird 1998).

2.4 Treatment regime

For sea lice (*Caligus* sp. and *Lepeophtheirus* sp.), teflubenzuron acts mainly by ingestion and then the chemical acts by interrupting larval and pre-adult moulting. It is effective against sea lice at a dose to salmon of 10mg/kg body weight per day for 7 consecutive days (Branson, 1995 and 1997).

Since teflubenzuron is active against the developing larval and pre-adult stages of sea lice, and less active against adult lice, it will be necessary to treat fish before adult lice appear, or are at least present in low numbers.

When used correctly, it is anticipated that Calicide will provide a treatment option by which the lifecycle of the sea lice can be broken. As a result, the duration between treatments may in fact be several months or longer. This has been observed at several sites in Norway where the product is in commercial use. A few Norwegian sites successfully used Calicide once during 1997 to remove all developing stages and the sea lice did not return during a further year's growth cycle (Ritchie, pers.com.).

However, lice from wild fish near the cages can reinfect the site at anytime and this cannot be controlled. In addition, if there are adult lice present on fish at treatment, then reinfection can occur and lice levels would return to those seen prior to treatment in about 8-10 weeks. Therefore on some occasions, fish farmers may require to employ prior chemical bath treatments and possibly repeat Calicide medication around 3 weeks after the initial in-feed treatment to ensure that the presence of adult lice or recruitment is controlled (Trouw 1998).

The final number of treatments per site will depend on these factors and could range from a single 7 day treatment to up to three 7 day treatments during the period May - October. The correct strategy for the use of Calicide will be made available to fish farmers and the industry by a product use strategy (Trouw, 1998).

The absorption of teflubenzuron from the gastrointestinal tract of salmon has been found to be poor with only around 10% of the administered dose retained by the salmon (Ritchie and Baardsen 1997, Jenkins 1996). This means that around 90% of the parent material will be released from fish via faeces in the period immediately following treatment and a further environmental input will be from any uneaten waste feed.

The main toxicity criteria for teflubenzuron to non-target species have been reviewed in expert reports (Jenkins 1995, Baird et al. 1997 and Baird 1998) .

Although teflubenzuron is relatively non-toxic to most marine species (e.g. fish, algae, shellfish), it is potentially highly toxic to any species which undergo moulting within their life cycle. This will therefore include some commercially important marine animals such as lobster, crab, shrimp and some zooplankton species.

3. FIELD STUDIES

Various field trials have been carried out in Norway (Myrvold 1997), Canada (CanTox 1997) and Scotland (Telfer 1998, McHenery and Ritchie 1998) to examine ecotoxicity and field effects.

3.1 Long term study

The first Scottish ecotoxicology trial, carried out during 1997 in Loch Eil (McHenery and Ritchie 1998), was a long term trial (1 year) to determine the fate and dispersion of teflubenzuron under normal treatment conditions using a low dispersion, "worst case site". This trial monitored effects on benthic biology and chemical residues were analysed in water, sediments and deployed mussels to assess fate and validate the sediment impact prediction model. During late 1998, this trial was extended (to day 654) with additional detailed studies on chemical residues in sediment, mass balance assessment, and benthic biology reported (Trouw, 1999).

At the original treatment date, a total of 19.6 kg of teflubenzuron was applied over a 7 day period to the treatment cage group which contained a biomass of 294.6 tonnes salmon.

Subsequent chemical analysis confirmed that measurable concentrations were generally not present in water after treatment and that levels in sediments were variable but followed the predicted dispersion model with measured levels of teflubenzuron extending initially to about 50m from cages in line with the main direction of current.

In the study, teflubenzuron was found to persist longer than 6 months, which was longer than expected and hence additional studies were commissioned by Nutreco at the request of SEPA and VMD. The predicted half-life of teflubenzuron in sediment was from 8 to 92 days depending on sediment type (Myrvold, 1997) and it had been expected that 90% of teflubenzuron should have been degraded within 6 months. The indication of a potential for longer persistence is attributed to the site being a "worst case" site already enriched and impacted by organic wastes with teflubenzuron being retained by binding with organic material (Trouw, 1999).

The results from long-term site monitoring finally reported a half life of 104 to 123 days (Trouw, 1999). From this data, **SEPA now intend to apply a half life of 115 days** as a decay factor when undertaking site loading calculations for consent applications or reviews.

Calculations from contour monitoring confirmed that although measurable levels of teflubenzuron were noted at distances up to 1000m in line with the main current flow, that by 645 days after last treatment, around 98% of the total load of teflubenzuron had been degraded or dispersed from the treatment site. In addition, importantly, no adverse effects were detectable on benthic biology or site crustacea and it was concluded that by later stages residual teflubenzuron was in a non-bioavailable form.

The benthic fauna at Loch Eil was typical of an impacted fish farm site where there is some degree of organic enrichment. It was concluded that, although there was evidence of an influence of teflubenzuron on existing benthic fauna in the intermediate area (around 50m from cages), there were no detectable adverse impacts on community structure and diversity including important key reworker species or on their bioturbation activity.

There was a lack of crustacea at the site and hence this precluded any opportunity to include monitoring effects on crustacea within the study. However, further re-examination of benthic biology, which focused on examining data on site crustacea (Trouw,1999), confirmed there was no detectable impact on crustacean population during the period studied.

3.2 Short term ecomonitoring study (STEMS)

In order to cover the issue of sensitive crustacea and to further monitor field effects, an additional short term ecomonitoring study (STEMS) was carried out at 3 locations in November 1997 viz. Loch Linnhe (south of Fort William) and Lochs Greshornish and Eishort (on Skye). This study included a novel biomonitoring technique whereby juvenile lobster larvae, which are a sensitive and significant species, were deployed on platforms at locations around cages.

It was concluded that juvenile lobster mortality was attributed to exposure to the medicated feed at 25m from cages, but that this effect did not occur at the 100m location. Importantly, it was confirmed that a moulting stage did occur during the period of lobster larvae deployment and exposure.

Sediment, mussel and water analysis along with the measurements from deployed sedimentation traps confirmed previous dispersion patterns where highest levels of teflubenzuron were detectable immediately under cages, decreasing with distance from the cages.

A significant finding was that there seemed to be some indication of resuspension and redistribution of sediment after a few weeks with consequent increased levels found in mussel tissues. This occurred even at the 100m site and it was concluded that this finding may have implications for the collection of shellfish near to cages.

Chemical analysis of samples collected on-site of indigenous crustacea was also undertaken. It was concluded that there was a risk that sediment dwelling crustacea, such as edible crab (*Cancer*) and possibly Norwegian lobster (*Nephrops*), may accumulate teflubenzuron from contaminated sediment. However, it is known that depuration and loss of teflubenzuron does proceed following initial exposure and uptake (McHenery, 1997) and hence levels may be lost from such species before toxic effects occur (moulting).

Environmental input was confirmed as being primarily from waste feed in the vicinity of the treated cages with a more widespread distribution arising from the dispersion of faecal matter extending to 100m from cages in the direction of current flow. These effects were as predicted by the dispersion model.

Sediment levels of teflubenzuron in the short term trial showed the expected trend of highest levels near the treated cages with detectable levels extending 100m from the cages at one month post treatment.

3.3 SEPA Audit

During the short term study period, SEPA carried out a site visit and audit to confirm compliance with the trial protocol and consent conditions. A set of inter-laboratory quality control samples was distributed (an analytical standard solution, spiked seawater and sediments) to all the analytical laboratories involved in the trial (Pirie 1998). This was carried out to check methods of chemical analysis and to assess the reliability of analytical data supplied since these were fundamental to the interpretation of effects.

The results of this audit and distribution of samples confirmed satisfactory performance of study protocols and of analytical quality between the laboratories involved in the trial (including SEPA West Region Chemistry laboratory). However, problems with some analytical measurements have been suggested for the full data set (Baird 1998) which reinforces the value of confirming analytical methodologies during trials. FFAG will continue to monitor the analytical quality of external and internal data.

For information, current analytical methods for teflubenzuron by LC-MS can achieve limits of detection of around 2 ug/kg (ppb) in sediment with a limit of quantitation of 20ug/kg and 2 ng/l (ppt) in sea water.

4. RISK ASSESSMENT

Environmental risk assessments by Baird et al.(1996) and McHenery (1997) used the process of comparing predicted environmental concentrations (PEC) with predicted no effect concentrations (PNEC).

Baird et al.(1996) concluded that the PEC exceeded the PNEC under cages, but that PNEC levels would not be exceeded 15m from cages. Since crustaceans are largely absent from this area, and evidence suggests that teflubenzuon is relatively non-toxic to sediment re-worker organisms such as polychaete worms, then environmental risks were low and considered acceptable.

Similarly, in the pre-trial risk assessment McHenery (1997) predicted that, although the sensitive lobster larvae deployed in the short term trial would be affected near to the treated cages (as was the case), that toxicity to species such as the mysid shrimp (*Mysidopsis bahia*) via exposure in the water column would be unlikely to occur. Predictions were that effects would occur in sediment reworking species in the intermediate area around treated cages and that impacts may persist for up to 6 months as teflubenzuron degrades.

Comparison of validated field results with PNEC values largely confirm these predictions (Telfer 1998). Sediment concentrations of teflubenzuron followed the pattern predicted from dispersion modelling.

Toxicity as measured by mortality to deployed lobster larvae indicated that some toxic impacts occurred within the cage impact zone (100m from cages) but with no detectable toxic effect beyond this.

Some uptake of teflubenzuron occurred in deployed mussels but it was concluded that little teflubenzuron remained after 28 days and depuration was rapid. In addition, no mortalities or adverse effects on chitin structures were detected (mussel hinge mechanism or byssus attachments).

Teflubenzuron was detected in tissue analysis of indigenous crustacea, particularly in larger sedentary crab species. It was suggested that although there were no toxic effects, that this finding would have an implication for the collection of crustacea near to sea cages during treatments.

5. CONCLUSIONS

5.1 Outcome of Risk Assessment

The main environmental risk of teflubenzuron to the marine environment is likely to arise from the deposition of fish faeces and waste feed to the sediments below and around treated cage fish farms. A large proportion (around 90%) of the treated chemical will be excreted as parent compound via faeces.

Although teflubenzuron is specific and of low toxicity to most non-target organisms there is an identified risk to crustacea near to cage sites at treatment.

There is a negligible risk of toxicity to organisms such as zooplankton in the water column (Baird 1998) despite the tendency of teflubenzuron to bind strongly to sediment and organic matter.

The half-life of teflubenzuron in sediment suggests that there is a moderate risk of build up in sediment through repeat applications, although the risk of this is reduced where fewer applications are required by correct use of the product strategy.

There has been additional detailed monitoring of the long-term dispersion patterns at the “worst case” site (Trouw, 1999). Appropriate sampling protocols were developed by Nutreco in consultation with SEPA and findings are being used to further validate

and modify predictive dispersion models. Around 98% of the original applied material had degraded by day 645.

Any implications arising from longer term spatial or temporal residue effects will be controlled by SEPA by the same process as for the initial consent, i.e. by application of the EQSs, by post authorisation monitoring and by site specific limits, or refusals to any further allowable treatments.

Sites which have consent to use Calicide will be required to include an additional benthic biological monitoring site, at 100-150m in line of main current, to their normal biological monitoring regime (SEPA, 1998). Post authorisation monitoring at high risk sites could also include the deployment of biomonitors (mussel and/or lobster larvae) and/or sediment chemical analysis.

SEPA will model sites and the initial consent will limit a maximum quantity of medicated product such that the sediments will remain within the two SEPA working sediment standards.

The allowable effects zone will be assessed to maintain levels such that the general sediment standard is not breached outwith the 100m area (or 150m in line of current). Risks of resuspension and movement outwith the initial effects zone are recognised, however it is considered that by the site specific consenting applied by SEPA that this presents a limited risk which will be further controlled by post authorisation monitoring, low bioavailability, the specificity of mode of action and SEPA review of any subsequent site applications.

5.2 SEPA standards for teflubenzuron in water and sediment.

A key issue that arises with this compound is the derivation of suitable sediment quality standards which consider the behaviour of the chemical, take account of site conditions and the risks of exposure of relevant sediment dwelling organisms to teflubenzuron at different cage locations (both the underneath cage community and the far field ecosystem).

The following standards will be applied by SEPA to control the use of Calicide. The standards have been refined from those issued previously (Revision 1.0 of SEPA Policy 29) and take effect as of 29 July 1999.

These standards are non-statutory SEPA working target standards which have been reviewed by WRc (Cole and Hedgecot, 1999) and, where appropriate, SEPA has taken account of WRc recommendations:

- **6.0 ng/l as an annual average** in sea water and **30 ng/l as a MAC**

Note: These standards were derived from chronic life-cycle data as measured concentrations on the 27 day MATC for *Mysidopsis* shrimp (Baird et al. 1997) and applying a x2 (for the annual) and a x10 safety factor for the MAC.

- **2.0 ug/kg dry wt/5 cm core depth** as a general sediment quality standard to be applied as a MAC to surface sediment (cores 5cm depth) outside the allowable effects area.

Note: This standard was derived from chronic life-cycle data as measured dry weight sediment concentrations on the 28 day NOEC to sediment dwelling amphipod *Corophium volutator* - a crustacean species - (Glass, 1997 and Aufderheide et al, 1999) using a x10 safety factor.

The allowable effects area, beyond which this standard will apply, is defined as 100m from the edge of cages (CSTT, 1994) - in effect the form of this area will be a large, shallow cylinder (5cm depth). During site depositional modelling calculations, this total surface area may be reshaped so that the sediment standard complies up to a maximum of 150m in any one direction. This is to allow some account to be made for sites where the deposition pattern is influenced by a strong main direction of current as compared to lower energy, multi-direction sites.

- **10.0 mg/kg dry wt/5cm core depth** as a standard applied as an average value within the immediate under cage impact zone defined as surface area under and around cages to a distance of 25m from cage edges.

Note: This standard was derived from sediment toxicity data (nominal concentrations) for *Arenicola* as a greater than value for the LC50 test and using a x1000 safety factor. The standard may be further refined by FFAG after the results of additional tests become available on more relevant impact zone re-worker species (e.g. *Capitella*). The standard will be applied as an average in areal terms (surface area of impact "footprint" to 5 cm depth) to take account of sediment volume.

5.3 Peer review

In view of the previous high profile for new sea lice treatments and since a similar approach has been followed by FFAG following initial SEPA authorisation for other recent sea lice treatments, FFAG placed a contract with the Water Research Centre (WRc) to carry out a peer review on our procedures (Revision 1.0) for the derivation of standards for teflubenzuron.

The peer review contracted to WRc (Cole and Hedgecot, 1999) reviewed the SEPA risk assessment of Calicide, and largely agreed with the appropriateness of the supplied data set and the values derived for quality standards for teflubenzuron. Minor changes and relaxation were proposed to the water column standard where an annual average and MAC were proposed and that no change should be made to the general sediment standard until information on organic matter levels of receiving sediments is more clearly understood. These recommendations have been accepted by SEPA.

It was considered that since only limited data was available on the suitability of the under cage standard that WRc could not assess the suitability of this standard. However, since this is a precautionary working standard, SEPA intend to continue to apply this for the purpose of under cage site loading assessment.

The comments from WRc have been taken into account in this present revision to Policy 29.

5.4 Consent Issues

SEPA requires information on any proposed use of a chemical to be provided in advance for new or reviewed consents to discharge medicines and chemicals from marine cage fish farms (SEPA, 1998). The applicant is also required to list the method of application, number of treatments and maximum treatment concentration.

SEPA's FFAG has developed (and will continue to review) standards which are applied to the ecosystem as appropriate for the particular route of environmental impact of different medicines or chemicals (water column, sediment, location and time where standards will be applied).

The use of in-feed treatments will be controlled using a mathematical model to predict concentrations in the environment around a fish farm. These concentrations will be compared with the relevant standards for the active ingredient and determination of consent will limit the use (or refuse) on a site-specific basis. Procedures for processing consents and the application of modelling are outlined in SEPA's Marine Fish Farming Procedures Manual (SEPA, 1998).

The controls on the use of Calicide will be monitored and reviewed at any time by FFAG in the light of new scientific evidence such as the development of improved predictive mathematical impact models and from the results of post-authorisation monitoring.

6. Recommendations

- The field trials on the use of the medicated feed Calicide as a sea lice treatment have shown limited risks and acceptable predicted environmental impacts.

- From these results, and from the evaluation of risk criteria, it is recommended that the use of Calicide be authorised by SEPA for consent to discharge initially for a 2 year period.

The consented quantity of medicated feed at each site will be determined by predictive models which will limit the impact on sediment for each additive treatment and maintain residual levels within the proposed EQSs. Consented quantities will be approved only for particular growth cycles. Thereafter application for reuse will require to be resubmitted to SEPA.

- FFAG will monitor and review as necessary the EQSs and their application, along with any site-specific consent issues..

- FFAG has taken account of the outcome of the WRc peer review of teflubenzuron standards.

- FFAG will continue to develop predictive models for the consent of in-feed sea lice treatments.

- FFAG will assist SEPA licencing teams during the consenting process.

- Applicants will be required to conduct post authorisation monitoring which will include incorporation of an additional benthic biology monitoring station at 100 - 150m into site monitoring protocols.

- Repeat medicated treatment within a growth cycle are controlled by detail in issued consents so that sites remain within the loading allowance. This will be by calculation using decay rates at the time of follow-on treatment.

- If Calicide treatments are required for future production cycles of stock, then these sites, if Calicide had been used previously, must conduct sediment surveys to measure residual levels of teflubenzuron in sediment. This data should be submitted to SEPA in advance of the proposed treatment to allow SEPA to assess baseline conditions for calculating additional teflubenzuron loading to sediments against the standards.

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GLOSSARY OF TERMS AND ACRONYMS USED IN THIS REPORT

ATC	Animal Test Certificate
FFAG	SEPA Fish Farming Advisory Group
EQS (sed)	Environmental Quality Standard in Sediment
IOA	Institute of Aquaculture, Stirling
LC_{50}	Concentration lethal to 50 % of test organisms as determined from a toxicity test
MA	Marketing Authorisation (formerly product licence)
MAC	Maximum Allowable Concentration
MRL	Maximum Residue Level
NOEC	No Observed Effect Concentration
Octanol : water partition coefficient	A measure of loss from water to organic solvent - gives an indication of capacity for adsorption and bioaccumulation by sediments or tissues
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
SOAEFD	Scottish Office Agriculture Environment and Fisheries Department
STEMS	Short Term Ecomonitoring Study
VMD	Veterinary Medicines Directorate
VPC	Veterinary Products Committee
WRc	Water Research Centre