Environmental Assessment for Import Tolerance for Tissue Residues of Teflubenzuron when used Outside the United States for the Control of Sea Lice in Farmed Atlantic salmon

1. **November 1, 2011**
   Name and Address of Sponsor:

   Skretting ARC
   Sjøhagen 3, P.O. Box 48
   4001 Stavanger
   Norway

Skretting is the world leader in the production and supply of feed for farmed fish. Skretting headquarters are in Stavanger, Norway. Skretting is not part of the pharmaceutical industry and does not manufacture pharmaceuticals. Total annual production of high quality feeds is approximately 1.3 million metric tons. Skretting has operating companies on five continents to produce and deliver feeds from hatching to harvest for more than 50 species of farmed fish. Skretting has fish feed plants in 12 countries with sales in over 40 countries, including the United States. Skretting is a part of Nutreco; an international company in the animal nutrition and fish feed markets. Skretting Aquaculture Research Centre (Skretting ARC) is the research and development part of Skretting, based in Stavanger, Norway.

2. **Description of Proposed Use:**

The proposed action is for FDA to grant an import tolerance that would establish a permissible level of teflubenzuron residues in food derived from farmed Atlantic salmon imported into the United States for human consumers.

Teflubenzuron (TFBZ) is a chitin synthesis inhibitor that interferes with the production of the chitin exoskeleton, and thus molting that occurs between life stages in the sea lice. This drug is currently approved for use in Norway, Ireland, the United Kingdom (UK) and Canada. It has a drug identification number and is currently used under an emergency drug release (EDR) in Canada (TFBZ marketing authorization is being updated in Canada and the drug continues to be available during this process.). Please note that the Bellona website (Bellona, 2009) is incorrect in stating that the drug is approved for aquaculture use in Chile.

The product is known under the market name Calicide in Canada, the UK and Ireland. It is called Ektobann in Norway. Calicide is 100 % micronized TFBZ. Ektobann consists of micronized TFBZ and the feed. Skretting markets only the complete medicated feed.

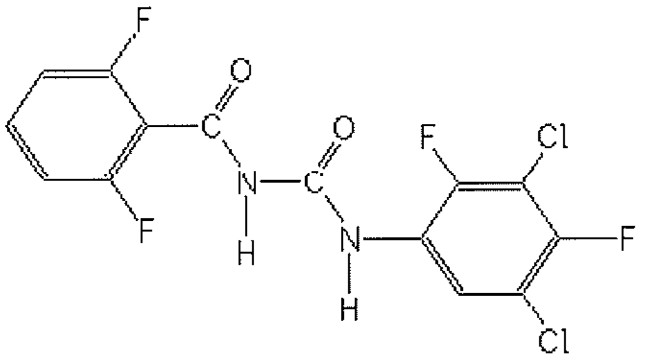
Teflubenzuron processed feed additive is obtained from approved manufacturers, added to feed by Skretting, and sold for use in farmed Atlantic salmon (*Salmo salar*) for the control of sea lice, parasitic copepods of the species *Lepeophtheirus salmonis*. The drug is added to feed at the dose of 2 grams active ingredient per kilogram of feed and offered to fish for 7 consecutive days. The therapeutic dosage is 10 mg TFBZ /kg body weight of fish per day for 7 days. Withdrawal times before slaughter range from 7-11 days (UK and Canada respectively) and 45-96 degree days (Ireland and Norway respectively). Degree days are the withdrawal period divided by the temperature of the water, for example at 10 °C, the withdrawal period in Ireland is 4.5 days and in Norway 9.6 days (Irish Medicines Board, 2010; Veterinary Medicines Directorate, 2010).

The product is most usually fed to small fish, where sea lice have a more dramatic effect on morbidity and productivity, usually many months before slaughter. TFBZ is most effective against early life stages of sea lice. Other products are available that are more effective against adult sea lice. TFBZ is to be used as part of an integrated pest management program that includes a number of other sea lice control products in combination with careful monitoring of the fish for infestation levels. TFBZ is unlikely to be used more than twice in the 18-24 months necessary to rear Atlantic salmon in net pens to market size. In any event, all feed is withheld from farmed Atlantic salmon for several days before slaughter.

The drug will not be marketed and fed to Atlantic salmon in the United States and consequently exposures in the United States will occur through importation and consumption of tissues from farmed Atlantic salmon. Therefore, there is not any anticipated disposal of the product, other than via excretion by consumers into wastewater. The proposed tolerance level is 500 µg/kg in the flesh and skin in natural proportions of processed farmed Atlantic salmon. This is the same level as set in Europe as a Maximum Residue Limit (MRL) (EMEA, 1999). The product is not approved for use in other species of farmed salmon.

3. Substances that are the Subject of the Proposed Use:

Teflubenzuron, CAS Number **83121-18-0**, [1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl) urea] is a substituted urea compound.



Teflubenzuron (TFBZ)

It is partially absorbed by fish from feed and rapidly and largely excreted intact via the feces. (EMEA, 1999).

Since the proposed action is to establish a maximum permitted level of teflubenzuron residue in the flesh and skin in natural proportions of imported farmed Atlantic salmon to be used for food for humans, it is this residue of TFBZ that is the subject of the proposed action. The proposed tolerance is 500 µg/kg muscle and skin in natural proportions. The limit of detection of TFBZ, the marker residue in salmon is 20 µg/kg.

Water solubility: 19 ug/L (pure water) (Tomlin, 1997), 9 ug/L water (Marsella et al, 2000).
3 ug/L seawater (Røn, 1997)
Octanol/Water Partitioning: Log Kow = 4.3-5.4 (Marsella et al, 2000).

4. Description of the Ecosystem at the Site of Introduction:

Three potential sites of introduction of TFBZ residues are assessed: (a) the ecosystems where the residues of TFBZ from consumed salmon might be introduced throughout the United States; (b) at aquaculture sites off the coast of New Brunswick, Canada; and, (c) the Global Commons. Two very conservative cases for potential quantities of TFBZ residues imported into the US as a result of the establishment of the proposed import tolerance are developed below. Additionally, potential concentrations of these residues entering into wastewater treatment facilities are estimated. TFBZ usage in New Brunswick and the potential for TFBZ to be introduced to the US from NB aquaculture usage is modeled as an example of the potential for TFBZ residues to move from one country, where use is approved, to a neighboring country, where use is not approved. Third, the potential for TFBZ to be introduced to the global commons is also estimated.

a. Ecosystems where TFBZ from consumed salmon might be introduced

Case 1: Assumptions: All processed salmon imported into the US will have been treated with TFBZ and all will contain TFBZ residues just below the level of detection of 20 µg/kg. This is a reasonable concentration of TFBZ to assume given the depletion pattern of TFBZ described in the 1999 EMEA summary (paragraph 23) and the likely husbandry pattern, described above, of treating small fish in order to gain the most economic effect in productivity gains and fewer losses. Additionally, there is the withdrawal time post-treatment as well as the industry practice of withholding all feed from salmon several (2-7) days before slaughter. Typically, fish will be treated many months before slaughter. The estimated population of the US is 307,212,123 as of July, 2009. (https://www.cia.gov/library/publications/the-world-factbook/geos/us.html).

If TFBZ is present at just below the detection limit for the analytical method (say 19.9 µg/kg of salmon tissue) and in the unlikely event that every kilogram of farmed salmon imported into the United States contains the residue (199,501,220 kg of farmed Atlantic salmon imported into US in 2008: USDA. 2009. (http://www.ers.usda.gov/data/Aquaculture/SalmonImportsVolume.htm). There would be a total of 3.97 kilograms of TFBZ residues imported into the United States each year.

A person consuming 0.25 kg salmon every day would consume and excrete a maximum of 4.975 µg of teflubenzuron each day. Each person in the US produces an average of 69 gallons = 3.8X69 = 262.2 liters of wastewater each day from domestic uses (US Environmental Protection Agency, 2002). Therefore, the salmon consumer would excrete TFBZ in wastewater at 0.019 µg/L (parts per billion, ppb). However, the quantity of farmed salmon imported in 2008 is only enough for about 798 million servings – about 2.6 servings for every person in the US per year. So, unless every person in a wastewater treatment facility collection area ate farmed Atlantic salmon on the same day, the concentration of TFBZ in the wastewater would be far lower. For example, if the population consumed salmon at random times throughout the year, this would approximate one consumer eating 2.6 servings over the course of a year.

(2.6 X 4.975)/(262.2 X 365) = 12.935/95703 = 0.000136 µg/L = 0.136 ppt

Given that wastewater is subjected to various treatments that vary by locale and that wastewater is also subject to dilution in receiving waters from runoff and groundwater, this concentration represents the highest level of teflubenzuron that could be present in a watershed receiving wastewater from a population of farmed Atlantic salmon consumers.

Case 2: All assumptions as in Case 1, except the concentration of TFBZ in all salmon is 40 µg/kg, the quantity present in salmon tissues at 24-35 days post treatment at water temperatures of 10 °C.

Total kg of TFBZ imported yearly = 40 µg/kg (199,501,220 kg) = 7.98 kg

Person consuming 0.25 kg of salmon daily excretes 0.25(40) = 10 µg TFBZ

Worst case excretion of TFBZ into wastewater from a person eating salmon daily = 10 µg / 262.2 L = 0.038 µg/L

Excretion from a population consuming 2.6 servings of farmed salmon per year = (2.6 X 10)/95703 = 0.000272 µg/L = 0.272 ppt

Both cases result in concentrations of TFBZ entering the waste water treatment system in the low parts per trillion range or lower. As the environmental fate information below shows, actually much less TFBZ would be expected to pass through wastewater treatment facilities, particularly those incorporating a biological treatment stage (e.g., activated sludge, trickling filters, lagoons), into receiving waters, as it partitions strongly into organic matter – the sludges produced by primary and secondary waste treatment.

Receiving waters from wastewater treatment facilities vary from small freshwater streams to large rivers to saltwater harbors and estuaries. Each has varying potential flows to dilute the introduced concentration further, as well as sediments and suspended organic material that would quickly remove TFBZ from the water column. See Appendix 1 (CanTox, 1997) for discussion on clearance of TFBZ from marine water column and sequestration in sediments.

b. Introductions at aquaculture sites abroad

Case 3: Canada as a model for potential movement of TFBZ residues from aquaculture, from a country where it is approved, to another country where such use is not approved.

Assumptions: Teflubenzuron was reintroduced to the Canadian marketplace in the fall of 2009. It is only used at the approved dosage and duration of 10 mg/kg Atlantic salmon body weight for 7 consecutive days. Aquaculture cage sites in New Brunswick range from 300-500,000 fish per site. For the purpose of this example we will choose the higher number of 500,000 fish. Biomass of salmon in a typical net pen facility increases as the fish mature and grow bigger. Assuming there are no mortalities during production so that the fish number remains constant and, assuming fish are treated in spring and fall to a maximum of 3 times during the production cycle in salt water; a typical facility would contain biomass of 62,500 kg (500,000 fish x 0.125 kg per fish) the first treatment, 500,000 kg (500,000 fish x 1 kg) the second treatment and 1,000,000 kg (500,000 fish x 2 kg) the third treatment. As mentioned above, the more usual treatment regime will be two treatments when the fish are still small.

The position of the Canadian (New Brunswick) saltwater aquaculture sites is shown on the attached map (Appendix 2). A higher resolution picture is included as an attached file (Fundy March 2011 map) but is not printed due to the size of the picture.

The drug is used at ≥9 °C in the absence of adult sea lice since adult sea lice do not moult and the drug therefore does not affect them. Teflubenzuron is not fed to sick or anorectic fish or in any circumstances in which the recommended amount of feed is unlikely to be eaten (for example low oxygen conditions or algal blooms). Treated Atlantic salmon are not slaughtered until at least 11 days after the last day of treatment. The maximum residue limit (MRL) for teflubenzuron is 0.3 ppm in salmonid muscle and 3.2 ppm in salmonid skin in Canada.

Feed medicated with teflubenzuron is made only when each individual prescription arrives at the feed mill. It then goes to the site and is fed according to label instructions. Unfed feed is highly unlikely. In the event that it is not possible to feed the Calicide it would be incinerated or put in an approved landfill.

Calculations:

Biomass of fish at treatment (kg) X 10 mg TFBZ /kg body weight X 7 days / 1000000 mg/ Kg = Total TFBZ (kg) applied at treatment

Treatment #	Biomass of Salmon (kg)	TFBZ Active Ingredient Introduced (kg)
1	62,500	4.375
2	500,000	35
3	1,000,000	70

c. Introductions into the Global Commons

21 CFR Part 25.60 implements an Executive Order on environmental impacts in the global commons. The underlying concern is whether the proposed action might indirectly contribute to environmental impacts outside the United States in countries less able to regulate damaging economic development or in the global commons – such as the oceans or air. In this EA, use of the product in Canada is used as a model for potential risks to the Global Commons.

Case 4: Canada as a model for potential introductions from aquaculture into the Global Commons

Residues from TFBZ used in aquaculture are very unlikely to reach Global Commons areas for several reasons. First, the locations of salmon net pens worldwide are in coastal waters. There is currently no economic incentive to build net pens in the Global Commons, miles away from the shore. Second, the quantities of TFBZ used in treatments are limited, as illustrated in Case 3 above, infrequent, and applied in feed rather than topically. Lastly, physico-chemical properties of TFBZ and ecomonitoring studies discussed below demonstrate that residues of TFBZ disappear rapidly from the water column, mostly by adsorbing to organic particles.

In sum, no residues of TFBZ are expected to be introduced into the Global Commons and no pathway of movement of residues from aquaculture sites in coastal waters miles away into offshore waters can be identified due to TFBZ physico-chemical properties and method of administration.

5. Fate of Introduced Residues:

TFBZ, when introduced into the environment, adsorbs tightly to the organic fraction of sediments because it has a moderate octanol:water partition coefficient, low water solubility and a short residence time in the water (Marsella, 2000, Scottish Environment Protection Agency (SEPA), Policy 29, 1999, Cantox, 1997, Røn, 1997). TFBZ typically biodegrades in sediment with a half life of about 35-100 days depending upon the type of sediment. (Bellona, 2009: SEPA, 1999). A later study found a longer half life of 104-123 days probably due to resuspension and settlement of the sediments (Telfer, 1999). Photolysis half-lives are measured in hours for TFBZ and this may serve as an additional removal mechanism for water column and sediment surfaces where light penetrates (Marsella et al, 2000). A brief review of the bioavailability and chemistry of teflubenzuron is provided in the Cantox, 1997 report. Ecomonitoring using caged lobsters and mussels confirmed the environmental fate predicted by direct measurement and by the chemistry of TFBZ. Effects were limited to moulting period of caged lobsters at 50 meters but not at 100 meters from treated salmon pens. Duration of exposure in the water column is generally 1 day but to no more than 3 days (Cantox, 1997).

a. Ecosystems where TFBZ from consumed salmon might be introduced

TFBZ introduced into wastewater due to the consumption and excretion of treated salmon by persons in the United States would partition into the organic sludge fraction in wastewater treatment facilities. Any escaping residues in effluent would partition into sediments. Biodegradation and, perhaps, photolysis would be expected to occur in sludges and at any sites of deposition outside the wastewater treatment facility.

b. Fate of residues at aquaculture sites abroad

Due to adsorption to organic particles, TFBZ is generally not present at measurable concentrations in seawater column one day after treatment at salmon farms except in the immediate vicinity of the cages which further indicates that it binds quickly to sediments (Appendix 1; SEPA, Policy 29, 1999). Ecomonitoring confirms that the potential effects area is limited to within 100 meters of net pens (Cantox, 1997). Photolysis and biodegradation have been shown to be mechanisms for removal of TFBZ from water column and sediment. (SEPA, 1999; Telfer, 1999; Marsella, 2000).

The potential for residues of TFBZ to migrate from treated salmon net pens to neighboring countries is limited in time, due to both the infrequent use of the product and the environmental fate mechanisms that rapidly remove it from the water column. Using the Canada-US example, residues at the US border would be transient and near the level of detection at most.

c. Fate of residues in the Global Commons

No TFBZ residues are expected to be introduced into the Global Commons, given the current configuration of salmon net pen aquaculture in coastal sovereign waters, miles from the Global Commons. In the event that net pen aquaculture relocates to the Global Commons, the same mechanisms of removal and degradation of TFBZ residues can be expected. One could speculate that the relative importance of photolysis in the removal of TFBZ residues from the water column would be greater in some offshore locations.

6. Effects of introduced TFBZ residues in the environment

Teflubenzuron is a medicine with a targeted and highly specific mode of action (interference with chitin synthesis) and a narrow band of toxicity. Teflubenzuron is safe to mammals, birds, fish, molluscs, echinoderms, coelenterates, algae and microorganisms. Many representative marine organisms, including other commercially important species have also been tested. These include but are not limited to; zooplankton, fish larvae, shellfish (oysters and mussels), worms, crustaceans in water and sediment, shrimps, crabs and lobsters. Numerous GLP field studies investigating the potential impact of both short and long term exposure on benthic community structure and function have been conducted. These studies addressed all the issues of importance for making decisions on the environmental suitability of the therapeutic use of the medicine and examined not only immediate and direct consequences to non-target organisms but also indirect consequences that may occur in the long-term or in other parts of the environment. Environmental risk assessments based on these studies were performed in Norway, UK, and Canada. See Appendix 1 and SEPA, Policy 29, 1999 as examples.

A review of acute and chronic ecotoxicity data is provided in Baird et al., 1996. Species that have chitin exoskeletons moult as they grow. It is this group that is potentially susceptible to the effects of teflubenzuron. Sensitivity to TFBZ varies among species, however. *Daphnia magna* and *Mysidopsis bahia* are indicators of the effects of teflubenzuron in freshwater and salt water invertebrates respectively. *Daphnia magna,* a freshwater invertebrate is sensitive to the effects of teflubenzuron at the time of moulting. *Daphnia magna* typically moult at 48 to 72 hours under laboratory conditions. One reported no effect concentration (NOEC) for *Daphnia magna* at 48 hours was 0.0063 μg/L. Other NOECs for survival and reproduction have been reported between 0.1 and 0.3 μg/L (Baird et al., 1996). In saltwater, a preliminary NOEC finding for survival, growth and reproduction of the mysid shrimp, *Mysidopsis bahia* was 0.037 μg/L. Adult and juvenile 7-day LC50 values of 0.059 and 0.057 μg/L, respectively were also reported (Baird et al., 1996).

Lobster larvae do not appear sensitive to teflubenzuron, LOEL $\geq$2.5 mg/L likely due to the short persistence time of teflubenzuron in water and its limited solubility (Cantox, 1997). Adverse effects on juvenile or adult lobsters are expected to be limited to the moulting period, localized within 50 m of the treated site and would decrease over time due to metabolism of the teflubenzuron within the sediments (Cantox, 1997).

To investigate long-term ecological effects, Skretting conducted several long-term GLP environmental monitoring field studies in the UK, Canada and Norway, some of which lasted 3 years. These studies examined the potential impact of TFBZ on benthic community structure and function and health of the benthic ecosystem. It was shown there was no discernible impact on benthic community structure and function even within close proximity of the treated cages. See Appendix 3 (Telfer, 1999) and SEPA Policy 29, 1999 as examples.

All the above studies were made available to, and reviewed by the regulatory bodies of Norway, UK, Ireland and Canada in order to determine conditions of safe therapeutic use of the drug in the culture of Atlantic salmon. Some of them are summarized on the Bellona Aqua Web website at http://www.bellona.org/aquaculture/artikler/Teflubenzuron. Summaries of others may be seen in Sepa, Policy 29, 1999. These ecotoxicology studies were conducted as part of the product registrations in several countries to devise safe conditions for use of the product and the FDA has access to these studies. However, they have to be regarded as confidential company information because if released to the public they could conceivably be used by others to register TFBZ in other countries.

The levels of TFBZ that might occur in receiving waters of the US resulting from consumption of farmed salmon food products are fully expected to be well below concentrations that might affect organisms in the environment. As also discussed above, presence of TFBZ is unlikely to be detected and would be transient due to the limited consumption of salmon in the United States.

a. Ecosystems where TFBZ from consumed salmon might be introduced

Treated wastewater may be introduced into either freshwater, saltwater, or esturine environments in the US. The daphnia and mysid NOECs cited below are the lowest concentrations of the studies conducted.

Environment type	Indicator Organism	NOEC or LOEC
Freshwater	*Daphnia magna*	0.0063 μg/L NOEC
Estuarine and Saltwater	*Mysidopsis bahia*	0.037 μg/L NOEC

b. Residues emanating from aquaculture sites abroad in neighboring countries and in the Global Commons

The relevant effects data to assess risk from residues crossing the US border from net pen treatment areas in Canada would be the mysid and lobster, as all such sites are present in saltwater coastal areas. The same indicator organisms would be appropriate for Global Commons areas, were they to receive any introduced TBFZ residues. As stated above, no exposure of the Global Commons to TFBZ is anticipated.

Environment type	Indicator Organism	NOEC/LOEC
Estuarine and saltwater	*Mysidopsis bahia*	0.037 μg/L NOEC
	Homarus americanus	≥2500 μg/L LOEL

7. Risk Characterization based upon the Exposures and the Hazards:

a. In the US environment.

Introductions of residues into the environment are limited by the infrequent use of the product in a species that is a minor part of the US consumer diet. Exposures will be at nondetectable levels due to the characteristics of the product; the low water solubility, partitioning into sludge at wastewater treatment facilities and short residence time in receiving waters. TFBZ rapidly disappears from water, due to its low solubility and high affinity for sediments and other organic material. Fate information indicates that biodegradation occurs in sediments. Photolysis occurs where light penetrates. Effects information indicates that aquatic crustaceans are the most sensitive organisms to TFBZ and that the threshold nominal concentration for such effects is at a much greater concentration than would be expected to even transiently occur in the environment as a result of the proposed import tolerance action. No effects are expected, even to the most sensitive organisms.

b. In the US and in other countries bordering countries where the product is used, or in the Global Commons.

There is limited potential for TBFZ residues to migrate away from approved sites of use or to cause effects near the sites of use,TFBZ is not expected to cause effects to the most sensitive indicator organisms. This has been confirmed by field studies.

Lobster larvae do not appear sensitive to teflubenzuron, LOEL ≥2.5 mg/L likely due to the short persistence time of teflubenzuron in water and its limited solubility (Cantox, 1997). Adverse effects on juvenile or adult lobsters are expected to be limited to the moulting period, localized within 50 m of the treated site and would decrease over time due to metabolism of the teflubenzuron within the sediments (Cantox, 1997).

TFBZ has been evaluated for environmental fate and effects under field conditions. Measured levels of teflubenzuron in the water were highest at the treatment site 1 day after treatment compared to stations 50 and 100 m offshore, 50 m inshore and the control site. Teflubenzuron was 0.0355 μg/L in water 3 m below the surface at the treatment site one day after treatment, between 0.01-0.03 μg/L 50 m offshore from the treated site and nondetectable at 50 m inshore, 100 m inshore and at the control site. Samples of water 3m below the surface taken at all sites 3 days and 32 days after treatment were below the limit of detection (Cantox, 1997). In saltwater, the NOEC for survival, growth and reproduction of the mysid shrimp, *Mysidopsis bahia* was 0.037 μg/L. The TFBZ levels measured in the water column at the site of treatment are below the NOEC for survival, growth and reproduction of *Mysidopsis bahia.* Teflubenzuron is not expected to impact *Mysidopsis bahia* in the water column.

Teflubenzuron concentrations in water taken 1 m above the sediments were more variable. Teflubenzuron was not detected at 1, 3 or 32 days after treatment at the control site, 50 m inshore and 50 and 100m offshore with two exceptions:
Day 1, 50 m offshore one sample was measured at 0.1287 μg/L and day 3 at 100m offshore one sample was 0.0262 μg/L. Teflubenzuron in water 1m above the sediments was detected at the treated site 1, 3 and 32 days after treatment, 0.01-0.02, 0.05-0.13 and 0.013-0.015 μg/L respectively.

From the above field studies, it is apparent that net pen aquaculture would have to be relocated into the Global Commons before exposures to TFBZ could occur. In the unlikely event that this occurred, effects would be transient and limited to chitin-bearing invertebrates in close vicinity to the net pens.

TFBZ residues are expected to be trapped in sediments below and in the immediate vicinity within 100 meters of treated salmon net pens (Cantox, 1997). In the Cantox ecomonitoring study, concentrations of TFBZ ranged from a maximum of 52.6 mg/kg dry sediment beneath the treated net pen, to 0.65 mg/kg dry sediment at 50 meters offshore, to 0.12 mg/kg dry sediment at 100 meters offshore. Sediment directly below treated net pens is not in the United States, however, lobsters and fish may visit the site and then range back and forth across nearby country borders. Nevertheless, the impacts of TFBZ use on sediment dwelling organisms in the vicinity of treated net pens will be limited in time and magnitude. TFBZ use in limited in time because the treatments are only periodic, approximately every six months. TFBZ residues that are trapped below net pens are limited in time due to biodegradation by microorganisms, half-life approximately 115 days (SEPA, 1999). The magnitude of TFBZ effects on benthic organisms is limited due to the selective nature of its mode of action, affecting organisms that synthesize chitin exoskeletons. Lobsters are economically important examples of such benthic feeding crustaceans. As discussed in the Cantox study, the LOEC for lobsters exposed through water is $\geq$2500 μg/L, LOECs for 7 day exposures through feed were 500 μg/kg dry feed for juveniles and 2000 μg/kg dry feed for adults. The level of TFBZ in feed for treatment is 2000 μg/kg. Eighty percent of caged lobsters directly below treated net pens, feeding on wasted treated feed, successfully moulted (Cantox, 1997). Scottish ecomonitoring studies using caged lobsters also led to the conclusion that effects on lobsters were limited to the 100 meter distance from treated net pens (SEPA, 1999). Fish are even less sensitive. Turbot larvae NOEC was found to be 10,000 μg/L (Cantox,1997). Thus, limited potential is expected for effects on lobsters visiting treated net pens and then potentially migrating (or ceasing to migrate) across a nearby national border. TFBZ, while bioavailable to lobsters and fish, does not appear to bioaccumulate through food chains and is quickly depleted from tissues after exposures cease (Cantox, 1997).

8. Description of Any Alternatives to the Proposed Use (including mitigations):

The use of TFBZ for the control of sea lice is itself an alternative to another product, emamectin benzoate (Slice). There is concern about growing resistance of sea lice to Slice and other sea lice medicines. TFBZ is another available drug to use as part of an integrated pest management program that could extend the usefulness of Slice and other sea lice medicines.

No mitigations, other than those in effect in various countries applying to the use of the product in salmon net pens, are contemplated for the setting of an import tolerance for TFBZ. The EA demonstrates that the proposed TFBZ use should not result in significant impacts >100 meters from treated net pens, and thus, should not cause effects in an adjacent country or the global commons. No significant adverse environmental effects are expected from setting the tolerance, the proposed action.

9. Preparers:

Julia Mullins, Researcher at Skretting ARC, Norway. Julia Mullins graduated from the Ontario Veterinary College, University of Guelph, Ontario with a Doctor of Veterinary Medicine in 1992 and from the Atlantic Veterinary College, University of Prince Edward Island with a Masters of Science in 1996. Her undergraduate years were spent at Dalhousie University attaining a Bachelor of Science in marine biology (Honours).

Julia Mullins has worked as a researcher in fish health at the Skretting Aquaculture Research Centre in Stavanger, Norway since February 2008. She practiced as a fish health veterinarian for private aquaculture companies (Pan Fish Canada, Heritage Salmon) with aquaculture sites on both coasts of Canada and in the US for several years. She has also worked for provincial government in Ontario as a fish health biologist and for the provincial government in New Brunswick as their fish health veterinarian. She is a former member of the Veterinary Drugs Directorate Expert, Health Canada advisory committee on aquaculture.

John Matheson, Consultant from Matheson Consulting, North Carolina, USA. specializes in FDA regulatory affairs for biotechnology products and, more generally, compliance with FDA environmental regulations implementing the National Environmental Policy Act (21 CFR Part 25).

From 1975 – 2006, he served in various positions at the US Food and Drug Administration, including Senior Regulatory Review Scientist in the FDA Center for Veterinary Medicine, specializing in environmental compliance for industry and agency initiated actions and in animal biotechnology, including transgenic animals and cloning. He prepared and helped defend environmental assessments and an environmental impact statement for numerous FDA proposals including prohibitions on the use of nitrofurans, subtherapeutic antibiotics in animal feed, gentian violet, sulfamethazine, enrofloxacin and others. He also reviewed hundreds of proposed animal drugs, drugs, and food additives for potential environmental impact and the applicability of FDA environmental regulations.

He chaired the CVM Aquaculture Coordinating Committee for many years. He co-chaired an international working group of the Organization for Economic Community Development (OECD) developing a Consensus Document on Atlantic salmon, to be used in the international environmental assessment of genetically engineered Atlantic salmon.

Matheson holds a Master of Science in Public Health from the UNC School of Public Health, Department of Environmental Sciences and Engineering (1975) and a Bachelor of Science in Biology, inducted into Phi Beta Kappa, University of North Carolina, Chapel Hill (1973).

10. Responsible Company Representative:

Dr. Alex Obach

Managing Director Skretting ARC

November 1, 2011

11. References:

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Fundy March 2011 map. Pdf.

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12. Appendices:

As mentioned earlier in the environmental assessment these ecotoxicology studies were conducted as part of the product registrations in several countries to devise safe conditions for use of the product and the FDA has access to all these studies. However, they have to be regarded as confidential company information because if released to the public they could conceivably be used by others to register TFBZ in other countries. As such, a summary of the studies is included in the appendices.

Appendix 1

EXECUTIVE SUMMARY

A chemical treatment developed by Nutreco Aquaculture Research Centre (ARC) has been proposed for use against salmon sea lice. The product, CME-134, is a powder that is coated onto commercial fish feed pellets. After ingestion, the active ingredient, teflubenzuron (TBZ), is absorbed in the gut of the salmon and subsequently distributed to the skin where the sea lice reside. The mechanism of action of TBZ involves inhibition of chitin synthesis, which prevents successful moulting of the sea lice, and ultimately leads to their death. Due to its mechanism of action, TBZ is effective at eradicating sea lice from salmon aquaculture sites, but also may have the potential to adversely affect non-target crustaceans in the neighbouring environment.

CanTox Inc. was requested by Moore-Clark, Canada and Nutreco ARC, Norway to conduct an ecomonitoring program and ecological risk assessment to address the potential for adverse effects of TBZ on non-target marine organisms living in the vicinity of a typical salmon aquaculture site in Eastern Canada. Non-target organisms, especially crustaceans such as lobsters and crabs, may be susceptible to the adverse effects of TBZ, which is released into the surrounding environment *via* residual feed pellets and salmon faeces. For this reason, an ecomonitoring program was designed to evaluate the potential fate and toxicity of TBZ to non-target marine organisms under normal sea lice treatment conditions at a typical salmon aquaculture site in the Bay of Fundy.

The short-term ecomonitoring field study commenced on July 4, 1996, with deployment of caged mussels and lobsters at specific locations in the vicinity of two active salmon aquaculture sites in the Bay of Fundy. Salmon at one of the aquaculture sites were given feed containing TBZ, according to the treatment protocol, while salmon at the other site were given regular feed. Mussels, lobsters, and samples of seawater and sediment were collected at specific distances and time intervals after treatment to monitor the fate of TBZ in the environment. In addition, groups of lobsters were observed for moulting success following treatment. Finally, the results of the ecomonitoring study and previous laboratory and field studies were incorporated into an ecological risk assessment to evaluate the potential for adverse effects in various groups of non-target marine organisms.

The results of the ecomonitoring field study and ecological risk assessment indicated the following:

Environmental Fate of TBZ in the Marine Environment:

- TBZ has a low solubility in seawater and was generally not present at measurable concentrations after day 1 post-treatment, except in water at depth (*i.e.*, close to the sediments) in the immediate vicinity of the salmon cages;
- The major sink for TBZ in the marine environment is sediments directly beneath the salmon cages, where it can persist for greater than 6 months after sea lice treatment;
- The distribution of TBZ in sediments was highly variable, likely due to the "patchy" deposition of residual feed and salmon faeces
- TBZ in sediments at varying distances from the treatment site appeared to be degraded over time to levels below detection between 1 and 6 months post-treatment;
- The zone of impact was confined to within 50 m offshore of the salmon cage site in the direction of dispersion, with very little impact between the salmon cage site and the shore;
- The duration of impact in water was less than 3 days post-treatment, while the duration of impact in sediments directly beneath the salmon cages was greater than 6 months post-treatment.

Bioavailability of TBZ to Marine Organisms:

- TBZ is bioavailable to marine organisms, such as mussels and lobsters, but due to apparent metabolism, TBZ does not appear to have the potential to bioaccumulate through the food chain;
- TBZ concentrations in mussel tissue and lobster tissue appeared to decrease spatially and temporally following treatment;

- TBZ residues in lobster tissue decreased rapidly following removal from the source of exposure.

Effects of TBZ on Moulting Success in Lobsters:

- Moulting success was reduced in lobsters deployed within 50 m of the treatment site, but was unaffected at 100 m offshore;

- More than 80% of the lobsters deployed directly under the salmon cages at the treatment site moulted successfully in both the field and the laboratory following exposure;

- Successful moults did not seem to depend on the moult stage of lobsters prior to treatment, however most of the lobsters which died during moult were in the later stages of premoult prior to treatment.

Potential for Adverse Effects in Marine Organisms:

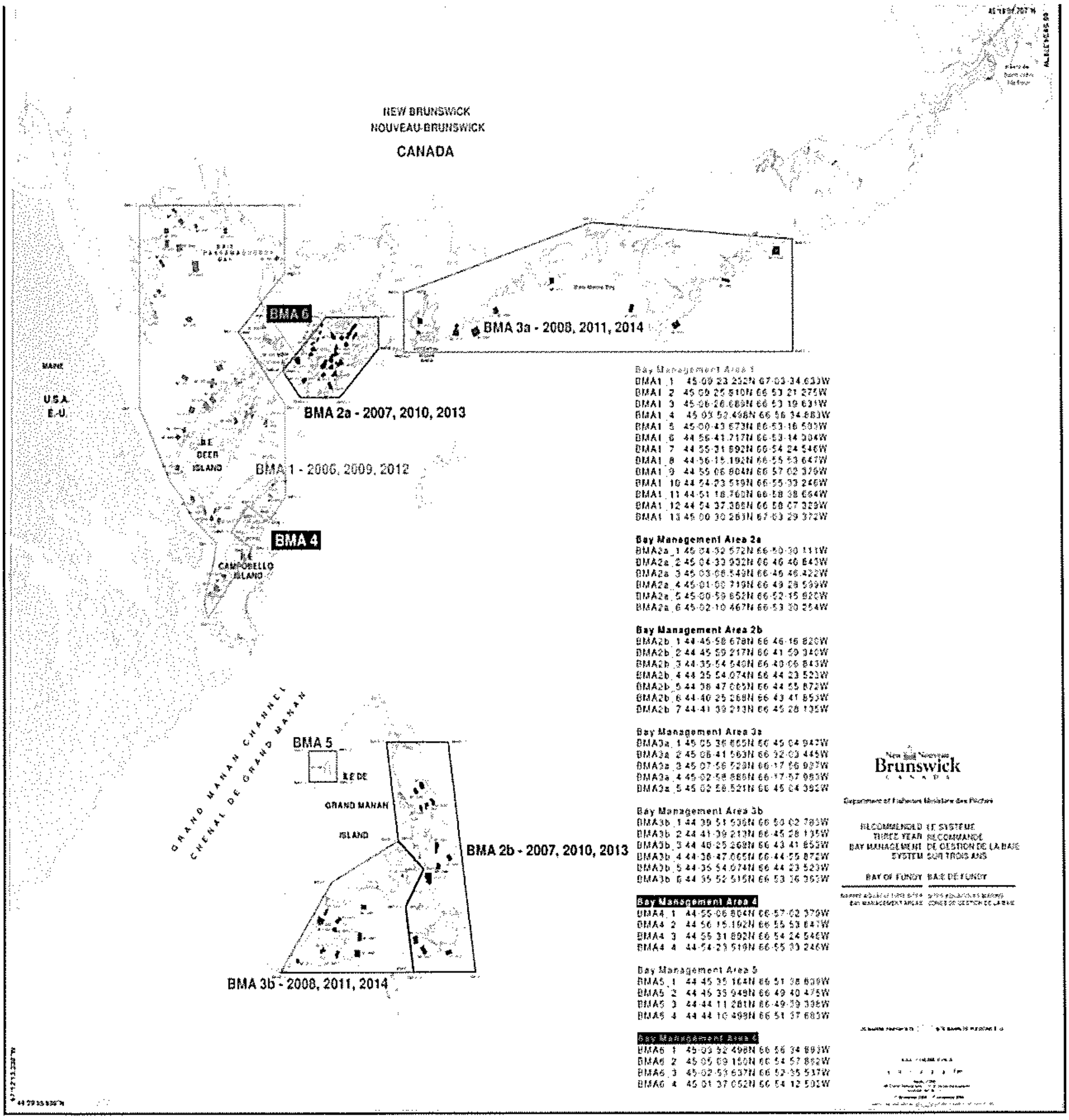
- Pelagic marine organisms, such as phytoplankton, pelagic crustacea, crustacea larvae and molluscs, would not be expected to be at risk of adverse effects following use of TBZ-treated feed at salmon aquaculture sites, since TBZ is relatively insoluble and has a short residence time in seawater;

- Unlike those organisms with a chitinous exoskeleton which grow through a series of moults, echinoderms increase in size by simply adding on to the existing material. It is therefore unlikely that echinoderms will be particularly sensitive to a pesticide which interferes with chitin synthesis and moulting;

- Benthic crustaceans may incur large exposures due to their close contact with and potential ingestion of faeces, residual feed and sediments, where TBZ tends to accumulate. These exposures, combined with the toxic action of TBZ on chitin synthesis, can cause adverse effects in benthic crustaceans. Based on the results of this assessment, however, the effects are expected to be limited to the moulting period, localized to within 50 m of the treated cage site, and would decrease over time following treatment or removal to a clean environment;

- Due to the reported metabolism in fish and the apparent metabolism observed in mussels and lobsters in the ecomonitoring study, the bioaccumulation potential of TBZ through the food chain is low and adverse effects in higher food chain organisms, such as marine fish, birds and mammals, are not expected.

Appendix 2


NEW BRUNSWICK
NOUVEAU-BRUNSWICK
CANADA
BMA 6
BMA 3a - 2008, 2011, 2014
BMA 2a - 2007, 2010, 2013
BMA 1 - 2006, 2009, 2012
BMA 4
U.S.A.
E.-U.
DEER ISLAND
CAMPOBELLO ISLAND
GRAND MANAN CHANNEL
CHENAL DE GRAND MANAN
BMA 5
GRAND MANAN
ISLAND
BMA 2b - 2007, 2010, 2013
BMA 3b - 2008, 2011, 2014
Bay Management Area 1
Bay Management Area 2a
Bay Management Area 2b
Bay Management Area 3a
Bay Management Area 3b
Bay Management Area 4
Bay Management Area 5
Bay Management Area 6
Brunswick
BAY OF FUNDY BAIE DE FUNDY

Appendix 3

ENVIRONMENTAL REPORT	**Distribution:** Confidential
Environment Group **Institute of Aquaculture, University of Stirling** **Stirling, FK9 4LA** **UK** **Tel: +44 1786 467878 Fax: +44 1786 472133**	**Project No:** ES-M/98/010b **Client:** Nutreco ARC
Report: Environmental Report	
Title: Analysis and interpretation of sediment samples from Loch Eil after treatment with 'Calicide' – November 1998.	

Summary

Sediment concentrations of teflubenzuron

- Teflubenzuron was still present in sediments after day 654, most of which is concentrated beneath the cages. There was a significant decrease in sediment levels at all stations between days 408 and 654.
- Calculations based on contour volumes suggest that between 1.27 and 2.56% of teflubenzuron entering the sediment was still present within the sampling area. Therefore 97.4 to 98.7% of teflubenzuron has been degraded or further dispersed by re-suspension over the 645 days since treatment. Assuming removal of teflubenzuron is due to degradation, these values approximate a half life in Loch Eil sediments of between 104 and 123 days.
- Concentrations of teflubenzuron are seen to be higher in the direction of main current flow.

Variation in macrobenthic communities

- Benthic macrofauna indicate similar trends in abundances and number of taxa between treatment cages and control sites, as shown previously up to day 408 (Telfer, 1998b) and no impacts on community structure that can be attributed to teflubenzuron.
- Species diversity shows little change throughout the study period from before commencement of treatment.
- There were considerable variations in numbers of key species such as *Capitella*, particularly at the control site, which has poor recruitment during the second year of the trial at both treatment and control sites. *Malacoceros*, however, illustrates typical annual recruitment at both treatment and control sites. These results suggest little influence on these community and species factors by the treatment.
- Multivariate analysis suggests a cycle of community change at sampling stations near both the treatment and control cages up to day 195, but a serial variation, for days 409 and 654, at stations near the treatment site. Though they have parallel production cycles, results between treatment and control are difficult to compare as they have considerably different fish biomass levels. The results may be accounted for due to the sediment near the lower biomass control showing more recovery from nutrient enrichment during its longer fallowing period (approx. days 330 to 450) than at shorter fallowing period of the higher biomass treatment site (approx. days 360 to 420).

Author: Dr. Trevor Telfer, *CBiol, MIBiol, MIEEM*	**Date:** 5 March 1999