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National Peer-review Meeting on Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments in the Bay of Fundy, New Brunswick

Réunion nationale d'examen par les pairs des recommandations pour définir l'exposition potentielle et les effets biologiques connexes issus des traitements des parasites et des agents pathogènes en aquaculture : bains contre le pou du poisson dans la baie de Fundy (Nouveau-Brunswick)

St. Andrews, New Brunswick
November 2-3, 2011

St. Andrews (Nouveau-Brunswick)
Du 2 au 3 novembre 2011

Meeting Chairpersons:
Jay Parsons and Ingrid Burgetz

Présidents de la réunion :
Jay Parsons et Ingrid Burgetz

Editor: Brent Scott

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March 2013 / **Mars 2013**

Canada

Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings include research recommendations, uncertainties, and the rationale for decisions made by the meeting. Proceedings also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

Avant-propos

Le présent compte rendu a pour but de documenter les principales activités et discussions qui ont eu lieu au cours de la réunion. Il contient des recommandations sur les recherches à effectuer, traite des incertitudes et expose les motifs ayant mené à la prise de décisions pendant la réunion. En outre, il fait état de données, d'analyses ou d'interprétations passées en revue et rejetées pour des raisons scientifiques, en donnant la raison du rejet. Bien que les interprétations et les opinions contenues dans le présent rapport puissent être inexactes ou propres à induire en erreur, elles sont quand même reproduites aussi fidèlement que possible afin de refléter les échanges tenus au cours de la réunion. Ainsi, aucune partie de ce rapport ne doit être considérée en tant que reflet des conclusions de la réunion, à moins d'indication précise en ce sens. De plus, un examen ultérieur de la question pourrait entraîner des changements aux conclusions, notamment si l'information supplémentaire pertinente, non disponible au moment de la réunion, est fournie par la suite. Finalement, dans les rares cas où des opinions divergentes sont exprimées officiellement, celles-ci sont également consignées dans les annexes du compte rendu.

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SUMMARY

Fisheries and Oceans Canada’s (DFO) Aquaculture Science Branch held a national peer-review advisory process November 2-3, 2011 at the St. Andrews Biological Station in St. Andrews, New Brunswick. The purpose of the meeting was to review and provide advice to the DFO Aquaculture Management Directorate in support of regulatory and policy decision making related to pest and pathogen management in aquaculture (see Appendix 1 for Terms of Reference). Specifically, the Aquaculture Management Directorate required science advice defining potential exposure and associated biological effects from sea lice bath treatments in the Bay of Fundy, New Brunswick. DFO scientists drafted three scientific working papers and meeting participants conducted impartial and objective scientific review of the papers. These proceedings summarize the discussions at the peer review. The conclusions and specific advice resulting from the meeting are contained in a Canadian Science Advisory Secretariat (CSAS) Science Advisory Report 2012/070 (DFO 2012).

SOMMAIRE

La Direction générale des sciences de l’aquaculture de Pêches et Océans Canada (MPO) a tenu un processus national d'examen par les pairs et de consultation les 2 et 3 novembre 2011 à la Station biologique de St. Andrews (Nouveau-Brunswick). La réunion avait pour but de réaliser des examens et de formuler des conseils pour la Direction générale de la gestion de l'aquaculture du MPO afin de faciliter la prise de décisions en matière de règlements et de politiques en ce qui concerne la gestion des parasites et des agents pathogènes en aquaculture (voir le cadre de référence de l'Annexe 1). En particulier, la Direction générale de la gestion de l'aquaculture avait besoin d'avis scientifique pour définir l'exposition potentielle et les effets biologiques associés des bains de traitement contre le pou du poisson dans la baie de Fundy (Nouveau-Brunswick). Les scientifiques du MPO ont rédigé trois documents de travail scientifique et les participants à la réunion les ont soumis à des examens scientifiques impartiaux et objectifs. Le présent compte rendu résume les discussions lors de l'examen par les pairs. Les conclusions et avis découlant de la réunion figurent dans l'avis scientifique du Secrétariat canadien de consultation scientifique (SCCS) 2012/070 (MPO 2012).

INTRODUCTION

Fisheries and Oceans Canada's (DFO) Aquaculture Science Branch held a national peer-review advisory process November 2-3, 2011 at the St. Andrews Biological Station in St. Andrews, New Brunswick. The purpose of the meeting was to review and provide advice to the DFO Aquaculture Management Directorate in support of regulatory and policy decision-making related to pest and pathogen management in aquaculture (see Terms of Reference in Appendix 1). This is the first meeting in a two part process examining the potential exposure and associated biological effects on key non-target organisms from aquaculture pest and pathogen treatments and the exposure and biological effects resulting from sea lice therapeutant bath treatments. Part two of this process will be broader in scope and include a full CSAS Science Advisory Process. Specifically, the Aquaculture Management Directorate required science advice defining potential exposure and biological effects from sea lice bath treatments in the Bay of Fundy, New Brunswick. In preparation for the peer-review meeting, two DFO scientists prepared three working papers which were distributed to confirmed participants (see Appendix 2) prior to the meeting. In addition seven of the meeting participants were responsible for conducting formal peer review of the working papers in advance of the meeting. At the meeting each paper was presented, reviewed, discussed, and then finalized before moving on to the next paper (see Meeting Agenda Appendix 3). The presentation format for each working paper began with the author providing the main points of the paper, highlighting results and conclusions. Each author's presentation was then critiqued by peer-reviewers. The discussion was then opened to all participants for questions and comments before finalizing conclusions and science advice related to the papers.

Welcome

The meeting chairs, Jay Parsons and Ingrid Burgetz, welcomed participants to the meeting which was followed by participant introductions. Ingrid Burgetz reviewed the meeting agenda, the (CSAS) science advisory process and the SAGE principles and guidelines (Scientific Advice for Government Effectiveness). Finally, the Terms of Reference were also reviewed which outlined the specific scientific questions being addressed in the research.

Context

Cultured salmon in aquaculture conditions are susceptible to infectious bacterial and viral diseases and infestations by parasites, such as sea lice. Sea lice are ecto-parasites which can pose a serious problem for the salmon aquaculture industry. Infestations on salmon result in skin erosion and sub-epidermal haemorrhage which, left untreated, can result in significant fish losses.

Therapeutants used to treat pests and pathogens in the aquaculture industry are considered either drugs or pesticides depending on the application method. Products applied topically or directly into water are considered pesticides; whereas, products delivered through medicated feed or by injection are considered drugs.

Regardless of the application method, pesticides and drugs used to treat sea lice are subsequently released to the aquatic environment which can impact other aquatic organisms and their habitat.

The potential for pesticide bath treatment releases to affect wild non-target organisms is determined by a combination of the exposure and toxicity of the therapeutant to the organisms.

Exposure is determined by both industrial discharge processes and natural transport and dispersal processes, including the behaviour of fish, while biological effects are determined by the concentration and duration of exposure and the organism's sensitivity.

This CSAS National Advisory Process involved a peer review of three research studies aimed at defining potential exposure and biological effects on non-target organisms from sea lice bath treatments in the Bay of Fundy, New Brunswick.

PRESENTATION HIGHLIGHTS OF WORKING PAPER
Transport and Dispersal of Sea Lice Bath Therapeutants from Salmon Farm Net Pens and Well Boats Operated in Southwest New Brunswick: A Mid-Project Perspective and Perspective For Discussion

Author: F. Page

This study focused on therapeutant transport and dispersal dynamics and the factors influencing the extent of exposure of non-target organisms following sea lice bath treatments using well boats and tarps.

Similar research related to pesticide transport and dispersal from bath treatments has been done in Scotland, incorporating basic assumptions to develop simplified models. The research results and conclusions presented herein are considered preliminary and will add to the body of knowledge in this area.

Pesticide transport and dispersal in the aquatic environment following bath treatments are influenced by a wide range of fixed and natural factors which affect the degree of exposure and biological impact on non-target organisms.

Therapeutant releases following tarp treatments may be near instantaneous, or spread out over tens of minutes to a few hours, depending on the degree of cage biofouling.

Based on dye dispersal studies, plume dispersions following tarp treatments were in general agreement with mathematical predictions, after the plume completely passed through the cage. In the near-field, cage infrastructure can enhance dispersion.

Plume transport distances are highly variable, and depending on site and current conditions, range from 200 m – 2 km, two hours following release.

The relative toxicity of a therapeutant over time and distance travelled will determine the degree of impact on non-target organisms in the near-field and far-field.

The physical characteristics of well boat discharges varies due to variations in the, discharge rate, discharge angle and direction.

Due to a combination of commercial operating protocols in southwest New Brunswick in which well boats remain at farm sites during discharge, and due to some vessels being configured with discharge pipes mounted on each side, fifty percent (50%) of well boat flushing discharges are directed away from the farm infrastructure and 50% are directed into fish pens; as such the exposure profiles from discharges directed into farm infrastructure affects exposure modelling capabilities.

Observations of discharges following well boat bath treatments are consistent with jet discharge theory.

Please see the Research Document for additional information related to this study.

Peer Reviewer Highlights

Presenter: D. Greenberg

The research was approached in a methodical manner, combining theory and practical experiments; however, it did not estimate the maximum potential harm associated with treatments needed to support effective regulatory decision making.

The greatest potential exposure occurs when therapeutant is released at full concentration during the initial discharge period. Increasing the flushing/discharge time (i.e., decreasing the flow rate) would introduce smaller volumes of therapeutant into the water at any one time, thereby increasing dilution and reducing exposure concentrations. It was acknowledged, however, that this approach would increase the overall exposure duration.

The peer reviewer also made a number of technical recommendations which the author agreed to address in the finalized research document.

Presenter: G. Bugden

The report successfully outlined the range of factors influencing potential exposure of non-target species to therapeutant bath treatments. The information should be distilled down to a maximum exposure range to provide a regulatory context. In addition, simplified advection equations should be included for both treatment types related to how a given patch of therapeutant moves. This may provide sufficient detail and would also be more enforceable from a regulatory perspective.

The near-field and far-field should be defined, and it would be beneficial to outline the distance at which discharge processes (e.g., pipe diameter, discharge velocity/direction) and tarp removal processes cease to significantly influence transport and dispersal dynamics. Depending upon the therapeutant toxicity, many site specific influencing factors cease to be important beyond a certain distance. To a large extent, the toxicity of the therapeutant, and thus the dilution required before the impact on the marine environment is considered to be negligible, will determine whether the near-field or far-field processes must be considered in a particular case.

Presenter: D. Stucchi

In his absence, Mr. Stucchi's review (see Appendix 4) was presented by Jay Parsons.

The peer reviewer provided a number of technical recommendations which the author agreed to include in the Research Document.

Open Discussion Highlights

Initial response by author noted that maximum potential harm was addressed in paper #3.

Initial discussions focused on whether target therapeutant concentrations (or effective treatment dose) were reached during tarp treatments, due to limitations with the fluorometer's detection capabilities and variability in the tarp volume estimates. Although treatment efficacy was beyond the scope of this meeting, it was suggested that the author outline the variability in initial mixing and treatment concentrations to account for the potential range in therapeutant concentration being introduced into the environment.

It was suggested that the author clarify the assumptions used and the relative importance of the factors influencing transport and dispersal. This includes differentiating between variability (highly variable or not) and uncertainty (not known).

The author should note the potential for benthic impacts and reduced diffusion when a plume encounters the benthos, due to certain well boat discharge pipe locations. For example, some well boats have discharge outlets located on the vessel bottom which force plumes down toward the benthos. Other well boats have side mounted discharge outlets which may result in the plume being directed toward cages. Plumes from these side mounted discharge outlets may also be deflected downwards toward the benthos when they encounter heavily biofouled cages.

Discussion around the following topics took place and additional comments to consider including in the Research Document, if within the scope of the paper, are:

- The plume shape at depth should be described as well as the influence this has on potential benthic exposure.
- The variability in vertical mixing (Z-axis) should be accounted for, either in absolute terms or relative to horizontal mixing (X and Y axes).
- The influence of the layout and proximity of other cages and farm infrastructure on plume dispersion.
- The role of therapeutant adsorption to organics in the water and how this influences dispersal estimates and therefore bioavailability.
- The rationale for why existing circulation models for southwest New Brunswick have not been incorporated into the analysis / modelling was raised and will be a focus in a future part 2 CSAS process.
- The potential cumulative exposure from multiple bath treatments per site and potential overlaps in treatments at multiple sites on any given day.
- The timelines associated with transport distances and the associated range of dilutions.
- It should be noted that plumes are generally elliptical in shape, and not circular, but site specific characteristics will result in differences in the magnitude of the major and minor axes. The plume shape will influence potential exposure of non-target organisms, depending upon their mobility and escape behaviour.
- It was recognised that there are differences between the therapeutant quantity required for tarp and well boat treatments and the potential exposure impacts. Due to the small volume of well boat wells, four treatments are required to treat each cage. Though the total therapeutant volume required for well boat treatments is less than for tarp treatments, four smaller releases result in different spatial and temporal impacts.
- The importance of physicochemical transformation of the therapeutant in the water column in predicting toxicity.

PRESENTATION HIGHLIGHTS OF WORKING PAPER
A Review of Potential Environmental Risks Associated with the Use of Pesticides to Treat Atlantic Salmon Against Infestations of Sea Lice in Southwest New Brunswick, Canada

Author: L. Burridge

This study reviewed therapeutants currently, or recently, used for controlling sea lice in the Bay of Fundy, New Brunswick including Salmosan® (active ingredient: azamethiphos), Paramove® (active ingredient: hydrogen peroxide) and AlphaMax® (active ingredient: deltamethrin) and assesses their risks to the aquatic ecosystem. The results and conclusions presented focus on three non-target species indigenous to the Bay of Fundy, including adult American lobster, *Homarus americanus* and all larval stages, and two shrimp species, the Mysid shrimp, *Mysis stenolepsis* and the Sand shrimp, *Crangon septemspinosa*.

The following is a summary of the research results:

Sensitivity is species and life stage dependent for all three therapeutants.

American lobster was consistently the most sensitive local species tested to AlphaMax® and Salmosan®.

There is evidence that time of year may affect adult lobsters' sensitivity to Salmosan®.

AlphaMax® is more toxic to lobsters and other invertebrates than Salmosan® which is more toxic than Paramove®.

Bi-weekly 1 hour exposures to Salmosan® at 5 and 10% of treatment concentrations (5 µg/L and 10 µg/L) resulted in significant mortality and reduced spawning success of surviving lobsters. Hence, there is lab-based evidence of potential *in situ* sub-lethal and delayed effects in adult American lobsters from Salmosan® exposures at below treatment concentrations.

Please see the Research Document for additional information related to this study.

Peer Reviewer Highlights

Presenters: V. Palace and W. Fairchild

The research paper discussed the relevant literature for the Bay of Fundy; it was noted that additional information is available for other species and geographic areas that could have been included.

The author should clarify that the study was laboratory-based, not field-based; subtle effects are not revealed by lab LC_{50} type testing or by short term studies done *in situ*. Longer term testing may reveal effects that have not been addressed in this study or in the scientific literature.

A comparison between the relative pesticide volume needed to treat one cage using each bath treatment method (tarped cage vs well boat) should be included to illustrate the total therapeutant subsequently released following each bath treatment method.

It should be specified whether the studies' conclusions are based on the pesticide formulation or the active ingredient.

The question was raised whether fish exhibit a stress response from being pumped into, and out of, well boats, and would that response make them more susceptible to subsequent infections by sea lice or other pathogens/pests?

It was suggested that additional background related to other available pesticides should be provided. For example, cypermethrin has 1/10th the toxicity of deltamethrin. Pyrethrins are also an option, especially in well boats where treatment can be better controlled to assure efficacy. In addition, they degrade faster and may be a more environmentally acceptable alternative.

It should be stated that it cannot be definitively said where the pesticides end up, how they accumulate, or what their potential impacts are. It was noted that two existing studies which suggest that the terrestrial use of these chemicals results in build-up in estuaries over large distances over time should be included in the literature review.

Open Discussion Highlights

Initial response by author, in response to the pesticide formulation or the active ingredient, noted that the non-active ingredients are proprietary and unavailable and that, from a risk assessment standpoint, using the formulation is more relevant than using only the active ingredient, as it is the formulation which is released into the environment.

In addition, author noted that while a number of valid points were raised, these were outside the scope of the Terms of Reference.

Discussion around the following topics took place and additional comments to consider including in the Research Document, if within the scope of the paper, are:

In terms of near-field effects, the question was raised regarding the amount of therapeutant absorbed by biofouling organisms. Similarly, is there evidence for benthic effects at previously treated sites?

Background should be provided on how a non-target species response to chemicals may depend on other stressors, such as moulting. For example, do organisms respond to chemical exposure similar to another environmental stressor that they are exposed to, or is the response chemical specific?

LT_{50} values should be included to better highlight the risk associated with different exposure times.

Where applicable, it should be specified whether results relate to measured or nominal concentrations.

Water temperatures used during the study should be included along with an explanation of how water temperature influences toxicity.

A species sensitivity analysis, using 5-6 species that take into account more sensitive and less sensitive species should be included.

The interplay between chemical properties and toxicities should be expanded upon to include:

- A comparison of toxicities between target and non-target organisms;
- A table outlining each chemical’s effectiveness at controlling each sea lice stage;
- A matrix outlining the timing of the presence of susceptible non-target species for use by management, and;
- A table describing and ranking the relative toxicities of the three compounds.

PRESENTATION HIGHLIGHTS OF WORKING PAPER
Estimates of the Effects of Sea Lice Chemical Therapeutants on Non-Target Organisms Associated with Releases of Therapeutants from Tarped Net Pens and Well Boat Bath Treatments: A Discussion Paper

Authors: F. H. Page and L. Burridge

This study combined field and model generated transport and dispersion estimates with laboratory estimated thresholds of lethality to determine potential scales of impact from sea lice pesticide bath treatments. The analyses consisted of three sea lice therapeutants: Paramove® (active ingredient; hydrogen peroxide); Salmosan® (active ingredient: azamethiphos), and; AlphaMax® (active ingredient: deltamethrin) and focused on three non-target species indigenous to the Bay of Fundy, including adult and larval stages of American lobster, *Homarus americanus,* the Mysid shrimp, *Mysis stenolepsis* and the Sand shrimp, *Crangon septemspinosa*.

Based on preliminary results, risk quotient ratios (exposure concentration: toxicity concentration) were calculated as indicators of the scale of impact associated with single treatments in the Bay of Fundy. Although this approach involves certain assumptions, uncertainties and variability, it does have scientific merit.

Further work is needed to develop a 3-D model to refine the exposure profile, and to predict the potential effects from multiple treatments.

Overall, the potential magnitude for impact is therapeutant specific: the degree and scale of impact increases from Paramove® to Salmosan® to AlphaMax®.

Actual biological impacts on exposed non-target species will be dependent upon the species and life stage, environmental conditions and the influencing factors outlined.

Please see the Research Document for additional information related to this study.

Peer Reviewer Highlights

Presenter: Peter Delorme

The research demonstrated a solid effort that takes a similar approach to Health Canada's Pest Management Regulatory Agency. The research went beyond the traditional Risk Quotient approach by determining time and distances to reach a specified effect endpoint concentration. This approach provides important context to the potential risks and may assist risk managers by defining a "zone of influence".

The LC_{50} and NOEC should be defined and it should be indicated how they were derived, as the NOEC values in the working paper are not technically considered NOECs (LC_{50} refers to the concentration where there is 50% mortality of the test subjects following a 1 hour exposure and $NOEC_{lethal}$ refers to the no observable effect concentration, based on lethality).

In terms of flushing times and LC_{50} and NOEC, the low pumping rate (600 m^3 h^{-1}) is likely unfeasible, due to safety concerns for salmon inside the well boat. As such, the question was posed whether it is worthwhile including this in the analyses?

More detail on assumptions, variability and uncertainties and their impacts on the results should be added. At a minimum, the relative importance of the major factors influencing risk should be identified.

It should be noted that the study focused on known sensitive groups of organisms that are used as surrogates [since protecting the most sensitive species, protects others as well] and that for a full risk assessment other representative taxa would be required for inclusion.

For well boats, the end-of-pipe risk assessment appeared to be a screening level risk assessment and very conservative. It should be emphasized that the scenario is a highly conservative assessment that overestimates exposure.

If a more conservative endpoint is needed then LC_{10} should be used rather than NOECs which are typically used for more chronic exposures.

Include a crude conservative estimate of dilution factors for AlphaMax® (deltamethrin) based on distance and time from the well boat. This would outline the zone of effect which is important for cumulative effects considerations and management practices.

Qualify that toxicity test results are currently based on nominal concentrations, not measured values which will be included in the final paper.

In terms of the environmental impact, the question was raised whether there is a need to distinguish between well boat discharges into cages and discharges into the open ocean.

Additional points that were raised include:

- The influence of cage size on risk, time and distance to specific concentrations;
- The influence of application timing, avoidance behaviour, and ecology of sensitive organisms on potential risks, e.g., the likelihood of sensitive lobster life stages being present during treatment;
- The influence of physical and chemical therapeutant properties on the potential for longer exposure and exposure to benthos, and;
- A description related to what this analysis indicates about potential best management practices.

Presenter: Bill Ernst

In his absence, Bill Ernst's review (see Appendix 5) was presented by Jay Parsons.

The peer reviewer provided a number of technical recommendations which the author agreed to include in the Research Document.

Open Discussion Highlights

The terminology related to low, moderate, and high potential exposure should be changed to more qualitative statements linking exposure and consequences.

A summary table outlining the time and distance to reach a non-toxic level for each of the pesticides, relative to each non-target species, including all life stages should be incorporated.

If possible, background should be provided related to why the prescribed dosage for sea lice is up to 1000 times higher than the toxicity dosage for some non-target species.

It should be qualified that the cage edge calculations for toxicity are conservative with respect to benthic life history stages due to entrainment and dilution.

Qualify why net cage plume calculations used a current speed value of 0.1 m/s.

In describing the vertical profile, clarify that this is based on measured data and not models or estimates.

Discussion should be included outlining that the exposure of benthic organisms depends upon site depth. For example, exposure may be restricted to the water column if a site is sufficiently deep because data suggests that the plume is restricted to the surface layers.

Another question was raised regarding public perception and possible lobster contamination: how long does it take for lobsters to get rid of the pesticide? In response, it was noted that there is some potential for bioaccumulation with pyrethroids; for example, past studies have shown that cypermethrin remains in lobsters longer than expected. It was added that Health Canada's Pest Management Regulatory Agency has set maximum allowable concentrations; however, from a mammalian (human) perspective, pyrethroids are non-toxic.

The author should account for the relationship between dispersal and exposure. As a plume disperses, although the concentration decreases, the exposure duration increases. Furthermore, the toxicity potential does not decrease in a linear fashion.

Qualify the potential for lobster exposure to pesticides, since only a small portion of a lobsters' life cycle (approximately 4-6 weeks) is spent in the water column. For adults, exposure would be limited to treatments that occur in shallow areas and by consuming previously exposed organisms.

Qualify that lobsters are not uniquely sensitive; other species, such as *Hyallela,* are equally as sensitive but they have not been included in this study.

CONCLUSIONS

The discussion and information reviewed at this CSAS process was the first opportunity to examine in-depth the scientific knowledge around the use of bath treatments on salmon farms in southwestern New Brunswick and the potential impact these treatments may have on non-target species. The following are key conclusions.

- The dispersion of bath treatments is highly dependent on site conditions and ocean currents, which will influence the distance the treatment chemical is transported as well as the length of time.
- The potential environmental exposure to bath treatment from a well boat depends on the angle and direction of discharge, pumping rate and duration, the treatment concentration and the receiving environment.
- American lobster was more sensitive to all three bath treatments than Mysid shrimp or Crangon. AlphaMax® was the most toxic bath treatment while Paramove® 50 was the least toxic.
- The potential biological impacts of bath treatments on non-target species depend on a variety of factors, such as the species, the life stage, and environmental conditions.

RECOMMENDATIONS

The following are potential research needs and priorities that have been identified:

- Field studies that assess both exposure and effects in parallel to laboratory toxicity studies with realistic exposure scenarios.
- Assessing exposure profiles of multiple treatments at single and multiple sites.
- The biological effects on non-target species from exposure to multiple bath treatments (i.e., sequential treatments) and multiple exposures to one treatment (i.e., pulsed treatments).
- Evaluation of the bioaccumulation kinetics of therapeutants resulting from single or multiple exposures.
- Assessment of interactions between therapeutants, the water column and sediments.
- Assessment of the effects on benthic organisms of therapeutants that may accumulate in sediments.
- Research on sub-lethal effects, as well as potential multigenerational impacts due to therapeutant exposure.
- Examination of biological effects on sensitive species with a range of ecological functions.
- Undertake a species sensitivity distribution (SSD) analysis.
- Identification of the temporal and spatial distribution of sensitive organisms (for use in risk mitigation measures).
- Identification and comparison of alternative approaches to combining exposure and toxicity data to assess biological effects and zones of influence.
- Development of *in-situ* effects and exposure monitoring methods and sample designs.
- Validation of plume pesticide concentrations with links to biological effects in the field - transferring laboratory based toxicity to field reality.
- Modelling of population level impacts.

REFERENCES

DFO. 2013. Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments in the Bay of Fundy, New Brunswick. DFO. Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/070.

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APPENDIX 1 – Terms of Reference

Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments

National Advisory Process

Phase 1: November 2-3, 2011
St. Andrews, NB

Phase 2: TBD

Context

In Canada, different aspects of fish pathogen and pest treatments are regulated under the *Food and Drugs Act, Pest Control Products Act* and the *Canadian Environmental Protection Act, 1999*.

Fisheries and Oceans Canada (DFO) has a mandate to protect fish and fish habitat under the *Fisheries Act*. Section 36 regulations are required to authorize deposits of substances that are deleterious to fish or fish habitat. Some therapeutants may fall into this category. Proposed regulations under the *Fisheries Act* would harmonize Fisheries and Oceans Canada, Environment Canada, Health Canada, and the Canadian Food Inspection Agency regulatory requirements to ensure that fish pathogen and pest treatment does not adversely impact fish and fish habitat and ensure that healthy aquatic ecosystems are maintained. Section 36 regulations typically restrict adverse effects outside a specified zone or area of impact.

As part of a separate, regionally-driven process, a Pacific Regional Science Advisory Process will be undertaken October 18-19, 2011, which will assess the environmental impact of the in-feed treatment of sea lice with the pesticide SLICE® at aquaculture facilities in British Columbia. The overall objectives of this Regional process are consistent with the overall objectives of the National process, and therefore the results will be considered within the overall National analysis. However, the Regional and National Advisory Processes are separate processes with distinct timelines and coordination.

Objectives

The Aquaculture Management Directorate has requested a national Canadian Science Advisory Secretariat (CSAS) science advisory process to provide peer-reviewed science advice to inform Fisheries and Oceans Canada's regulatory and policy considerations related to pest and pathogen management in aquaculture. The information and advice produced from this CSAS review will also be available to feed into future Health Canada environmental risk assessment processes for aquaculture pathogen and pest management products.

Specifically, science advice related to the exposure of non-target organisms to aquaculture pest and pathogen treatments and whether that exposure may result in biological effects has been requested.

In light of ongoing research and the current state of scientific information, in order to achieve the stated objective, a phased approach to providing this advice will be undertaken.

Phase I (November 2-3, 2011)

Phase I will be a national, internal to DFO, CSAS meeting to review the research results and analysis from the Program for Aquaculture Regulatory Research (PARR) funded projects related to sea lice bath treatments in the Bay of Fundy. The results will be used to support management and policy considerations for a risk tolerance/risk management approach to “no adverse effects to non-target species are occurring outside treatment areas” for aquaculture pest and pathogen treatments.

The Phase I CSAS meeting will address the following **specific questions**:

1. What are the factors that may influence the extent of exposure of non-target organisms to sea lice bath treatments (well boat and tarp application)? In order to address this question, a literature review of transport dynamics and dispersion in general is required, as well as an analysis of the dispersion and dilution of bath treatments in the Bay of Fundy specifically and the resulting model.
2. What are the known biological effects of hydrogen peroxide, azamethiphos and deltamethrin on key non-target organisms? Known biological effects may include lethal, sub-lethal, and behavioural impacts.
3. Using the Bay of Fundy experience as a model, what are the predicted biological effects on key non-target species (i.e., how does the exposure profile, including dilution and duration, map to the known biological effects from exposure at that concentration)?

Phase II (Fall/Winter 2012)

Phase II will be a full CSAS Science Advisory Process aimed at addressing the potential exposure and potential associated biological effects on key non-target organisms (including, where possible, consideration of population-level effects) from aquaculture pest and pathogen treatments.

Following Phase I, nominations for the Phase II CSAS Steering Committee will be sought.

The exact scope and specific questions for Phase II will be determined by the Steering Committee based upon outputs from the CSAS bath treatment meeting (Phase I), the Pacific SLICE Regional Advisory Process as well as ongoing regulatory policy requirements outlined by DFO Aquaculture Management.

Expected Publications

For the Phase I initial national CSAS meeting, research documents, and proceedings will be produced and published on the CSAS website. A science advisory report will be produced if determined appropriate, by the Steering Committee, based on the state of available scientific evidence.

As part of the full Phase II CSAS advisory process, research documents, a science advisory report and proceedings will be produced and published on the CSAS website.

Participation

1. **Phase I CSAS Meeting**: participation will likely be limited to experts within the federal government due to the limited nature of the available scientific information and on-going nature of the research. Participation will include Fisheries and Oceans Canada Ecosystem and Oceans Science experts from all Regions, Aquaculture Management and Habitat Management, and experts from other federal governmental departments.
2. **Phase II Full CSAS Advisory Process**: participation will be expanded to include academic experts, provincial experts, industry representatives, First Nations and wild fishery stakeholders, and environmental non-governmental organizations.

APPENDIX 2 – Participant List

National CSAS Review to Define Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments

Date: November 2 and 3, 2011
Location: St. Andrews Biological Station, St. Andrews, NB

#	Participants	Role
1	Bakker, Jiselle	Participant
2	Bartlett, Graham	Participant
3	Bugden, Gary	Reviewer
4	Burgetz, Ingrid	Co-Chair/Steering Committee Member
5	Burridge, Les	Author/Steering Committee Member
6	Chang, Blythe	Participant
7	Cline, Jeff	Participant
8	Cooper, Andrew	Participant
9	Cooper, Lara	Participant
10	Couillard, Catherine	Steering Committee Member
11	Delorme, Peter	Reviewer
12	Drozdowski, Adam	Participant
13	Fairchild, Wayne	Reviewer/Steering Committee Member
14	Fife, Jack	Participant
15	Greenberg, David	Reviewer
16	Haigh, Susan	Participant
17	Hamoutene, Dounia	Steering Committee Member
18	House, Nancy	Support
19	Johnson, Stewart	Steering Committee Member
20	Losier, Randy	Participant
21	Lyons, Monica	Participant
22	Martin, Jennifer	Participant
23	McGladdery, Sharon	Participant
24	Page, Fred	Author/Steering Committee Member
25	Palace, Vince	Reviewer/Steering Committee Member
26	Parsons, Jay	Co-Chair/Steering Committee Member
27	Perry, Geoff	Participant
28	Porter, Ed	Steering Committee Member
29	Quinn, Tammy-Rose	Participant
30	Ratsimandresy, Andry	Participant
31	Robinson, Shawn	Participant
32	Rouleau, Claude	Participant
33	Scott, Brent	Support
34	Scouten, Sarah	Participant
35	Waddy, Susan	Participant
36	Walker, Sherry	CSAS rep
37	Wong, David	Participant

APPENDIX 3 - Agenda

National CSAS Review to Define Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments

Location: Conference Centre, St. Andrews Biological Station,
St. Andrews, New Brunswick
November 2 and 3, 2011

Co-Chairs: Jay Parsons and Ingrid Burgetz

Day 1 – November 2, 2011

Time	Agenda Item	Presenter
8:15 – 8:30	Welcome and Introduction	Jay Parsons / Ingrid Burgetz
8:30 – 8:45	Review Agenda, Housekeeping and CSAS Overview and Meeting Procedures	Jay Parsons / Ingrid Burgetz
8:45– 9:00	Review Terms of Reference	Jay Parsons / Ingrid Burgetz
9:00 – 9:30	Presentation of Working Paper – Potential Exposure: a) Transport dynamics and dispersion influencing exposure of key non-target species b) Bay of Fundy experiences and resulting model	Fred Page
9:30 – 10:30	Reviewer Presentations and Author Response	David Greenberg, Gary Bugden, and Dario Stucchi
10:30 – 10:45	**Break**	
10:45 – 12:00	Open Discussion	
12:15 – 1:15	**Lunch**	
1:15 – 1:45	Presentation of Working Paper – Potential biological effects of bath treatments on key non-target organisms	Les Burridge
1:45 – 2:45	Reviewer Presentations and Author Response	Vince Palace and Wayne Fairchild
2:45 – 3:00	**Break**	
3:00 – 4:15	Open Discussion	Jay Parsons / Ingrid Burgetz
4:15 – 4:20	Summary and Adjournment	Jay Parsons / Ingrid Burgetz

Day 2 – November 3, 2011

Time	Agenda Item	Presenter
8:15 – 8:30	Introduction	Jay Parsons / Ingrid Burgetz
8:30 – 9:00	Presentation of Working Paper – Potential Exposure and Biological Effects Analysis	Fred Page and Les Burridge
9:00 – 10:00	Reviewer Presentations and Author Response	Peter Delorme
10:00– 10:15	**Break**	
10:15 – 12:00	Open Discussion	Jay Parsons / Ingrid Burgetz
12:00 – 1:00	**Lunch**	
1:00 – 3:00	General Discussion and Developing Science Advisory Report	Jay Parsons / Ingrid Burgetz
3:00 – 3:15	**Break**	
3:15 – 4:15	General Discussion and Developing Science Advisory Report continued	Jay Parsons / Ingrid Burgetz
4:15 – 4:30	Conclusions and next steps	Jay Parsons / Ingrid Burgetz

APPENDIX 4 – Peer Reviewer Comments from Dario Stucchi

Review of Page et al. CSAS working paper by D. J. Stucchi - 2011-11-01
Transport and Dispersal of Sea Lice Bath Therapeutants from salmon farm net pens and well boats operated in Southwest New Brunswick: a mid-project perspective and perspective for discussion.

CSAS questions posed for Page et al.

1. What are the factors that may influence the extent of exposure of non-target organisms to sea lice bath treatments (well boat and tarp application)? In order to address this question, a literature review of transport dynamics and dispersion in general is required, as well as an analysis of the dispersion and dilution of bath treatments in the Bay of Fundy specifically and the resulting model.

CSAS questions for companion reports.

2. What are the known biological effects of hydrogen peroxide, azamethiphos and deltamethrin on key non-target organisms? Known biological effects may include lethal, sub-lethal, and behavioural impacts.
3. Using the Bay of Fundy experience as a model, what are the predicted biological effects on key non-target species (i.e., how does the exposure profile, including dilution and duration, map to the known biological effects from exposure at that concentration)?

General Comments:

- This manuscript reports on "… work in progress…" and "... not to be considered as complete ...consideration ... of the data collected by the authors." My contribution is not so much a review as comments to consider (or not) for discussion and suggestions for further analysis and interpretation of the data, modelling approaches and alternate disposal methods.
- It is evident that many individuals have contributed and will continue to contribute to this large body of work. This is a complicated issue that is important to management of the fish farming industry and the fisheries. As an outside observer it would be informative to know the extent of the studies conducted to date and the plans for further studies, models and analyses of the data and measurements collected.
- One of the key results from this work should an understanding or ability to model/predict or determine the concentration of the therapeutant in time and space after a release to the receiving environment. This manuscript reviews some of the theory governing the spread and development of a plume of inert tracer in an idealized environment. Some preliminary comparisons of observation and theory are presented, and while some results are encouraging others are less so. There needs to be more effort directed to the presentation of the observed concentration of the tracer in the plume and a comparison of these observations with theory and/or models.
- Modelling is mentioned in the question for this CSAS contribution, but in this mid-project report modelling has not been addressed. Modelling may be the only viable approach when the theoretical approach is limited by simplifying assumptions and idealized conditions, and field observations are too costly to maintain in an ongoing or operational mode. Models of circulation of SW New Brunswick waters have been well developed and can be used to provide the basic current structure for the modelling of the therapeutant transport and dispersal away from the farm site. However, much

more is required to develop a credible model of the fate of the therapeutant plume and some of these requirements have been mentioned in the manuscript. The farm related factors that determine how quickly the therapeutant leaves the net pen need to be understood and parameterized if they are included in a model. The influence of net mesh size and degrees of obstruction by biofouling on flushing rate of the net pens should be amenable to quantification. The alteration of the ambient flow regime by other nets and farm structures will require additional and finer scale modelling of the flow in the vicinity of the farm. Modelling of the transport and dispersal of pollutant plumes and jets in the marine environment is not new and existing models/modules should be reviewed and assessed. Existing model/modules may require modification before they are coupled to or driven by the basic circulation model.

- The characteristics of the therapeutants in the marine environment have not been discussed in this manuscript nor have biological effects of the therapeutants on organisms in the region. Properties of the therapeutant such as decay time, affinity for to particulate material or absorption by organic solids, etc., will influence the nature and scales the biological effects and fate of therapeutant. Some of these issues are addressed in questions 2 and 3 of this CSAS process but they feedback to this first question. For example, if the therapeutant binds to organic solids either on the bottom or in the water column then the scope of the study should include consideration of the effects of the therapeutant on benthic organisms and the fate of the therapeutant in the benthic environment. Another example, if significant adverse effects on some organisms occur at very low concentrations or exposure levels and the therapeutant is long lived then the potential area and time scale of the problem could increase significantly from a few hours and kilometres to 10's of hours and kilometres.

Specific Comments:

Numbering of equations would greatly help the reader.

Transport and Dispersal from Tarped Cages

Page 3, par. 2, last sent.:
The injection of air/oxygen should produce some mixing.

Par. 4:
How is the therapeutant pumped into the net cage? Single or multiple point discharge or diffusers?
Do you assume thorough mixing or do you know that it takes 10 minutes to become thoroughly mixed?

Par. 6.
The ambient current velocities will be an important factor driving the speed with which the therapeutant leaves the cage system. Farm related factors such as cage configuration, cage size, net mesh size and degree of biofouling will also determine how quickly therapeutant is flushed from the cage by the ambient currents. Also the time taken to drop the tarps and pull them underneath the cage net may play a role in the timing of the release.

Page 6.
"Initial" means concentration of the therapeutant inside the cage once injection of the therapeutant has stopped and it is thoroughly mixed?

Par. 2
"assumed"? Surely you know the sizes of the cages and the depth of the tarp.

Par. 3:
Assuming a cubed shape seems an unlikely overestimate for the maximum volume. A cylinder with depth hc is a better estimate of the maximum volume and reduces the range in volume and therapeutant concentration estimates.

Page 7. par1:
How much leakage is there from a completely tarped net cage? Is leakage an issue?

Page 17, par. 2, sent. 3:
Sentence does not make sense -, rewrite. “Under these assumptions …”

Last sentence:
Time scales for depths of 23 to 30 m are from4 to 8 hrs for Kz = 0.01.

Page 18, par. 1:
Time scale for depths of 20 to 30m are reduced to 0.4 to 0.8 hrs for Kz =0.1.

Page 20, par.2, sent. 3.
“The predicted dilutions …” Which predictions are these?

Page 23, 2nd last sentence:
Why was dye constrained to top 5m? Vertical mixing depth in Table 3 suggest dye should have mixed more that 10m in one hour for Kz =0.01. Was stratification present?

Last sentence:
Can you compare measured dye concentration within the plume with predicted concentrations?

Page 27, par. 2:
“The center of mass…” Is this calculated by taking into account the concentration of dye or just the centroid of the patch outline?

Transport and Dispersal from Well Boats

p. 36, pars. 1 &2:
It appears from Fig 12. that the discharge from the well boat (Colby Perce?) is at or near the surface and directed horizontally. In this case the development of the jet will be constrained by the sea surface and the theory presented will need to be modified. The author acknowledges this later in the paper (p. 37 par. 2) but does address this further.

Discharges directed vertically or at angle of 45° downward will most probably impinge on the bottom as most farm sites are located in water depths of 20 to 30 m. Once again the theory presented for the dilutions of the therapeutant in the jet is not applicable and requires modification. Furthermore in the case of vertically directed jet buoyancy of the jet may need to be considered.

par. 3, line 3:
change to “... by a factor of ten along the centre line of the jet when..”

par. 3, sent. 2:
The concentration of the therapeutant at a distance x will actually be much less than Cmax (the centre line concentration) because of the exponential decay in the radial concentration.

p. 37, par. 1, 5th line
Overestimate should be underestimate.

p. 40, caption:
Replace “thin black line” with “open black circles”.

APPENDIX 5 – Peer Reviewer Comments from Bill Ernst

Comments from Bill Ernst on Fred Page and Les Burridge Discussion Paper: Estimates of the effects of sea lice chemical therapeutants on non-target organisms associated with releases of therapeutants from tarped net pens and well boat bath treatments: a discussion paper.

1. I am very supportive of this effort and think it goes some distance towards producing an estimate of risk to non-target organisms in the marine environment from sea lice bath chemical treatment. I believe the approach taken by Fred and Les is sound in principle and evaluates the information they have produced in a thorough manner. The conclusions are overall supportable by the arguments and will be of benefit to risk managers in making decisions on the use of such chemicals. I do have a few comments on the technical aspects of the paper.

2. The discussion is based almost exclusively on the results of the recent research conducted by the Page and Burridge teams. While that is an impressive dataset and I believe represents a substantive portion of the data that are available, the analysis might be improved by inclusion of additional published literature, in particular the hazard assessment literature. As an example the hazard assessment endpoints selected could be evaluated against other data in a species sensitivity distribution analysis, which is a common risk assessment technique and could further justify their selection of hazard endpoints. Notably the data of Fairchild et al. 2010 could be used.

3. Lethality may not be the most important endpoint to use in such an analysis and sublethal endpoints such as reproduction or paralysis may be as important from an ecological perspective as mortality, again see for example Fairchild et al 2010. It should also be pointed out that a mortality endpoint which is expressed as central tendency of a population (50%) still means that 50% of that population dies – which is not a conservative assessment technique. There should also be some caution given when making statements that the LC 50 based ratios indicate that particular organisms are unlikely to experience mortality unless the mortality curves are presented and evaluated further. For example a LC50 ratio of 0.8 for larval lobster to Paramove® may not mean that substantial effects in the population may not occur. The methods used to derive the NOEC used in this paper are no doubt reported in the Burridge paper referred to; however, it would be useful to see that calculation in this document.

4. The Introduction indicates that skirt treatments are no longer used, which is true, but there is no reason that I am aware of that they could not be used again in Canada in the future and perhaps a risk analysis should also be made for that method of treatment to inform the management decisions.

5. I believe one of the more important outcomes of the paper to be a relative ranking of risk of alternatives for the treatment of sea lice. For ease of comparison, it would be nice to see the relative risks of each chemical presented in a single table for each species perhaps as a factor of the lowest toxicity chemical.

6. The last sentence of the fourth paragraph indicates that duration of exposure is not explicitly considered, which is not quite true. The 1hr toxicity data is compared with the approximately 1 hr exposures in the containment, which is appropriate. In addition the 1 hr data are realistically compared with the immediate dispersion concentrations. The 24 hr and 10 d toxicity data are more conservative for single cage releases; however, they may become more important for multiple release scenarios as they are developed. The last two sentences of the last paragraph of the Toxicity of Therapeutants section should also be further explained since it is not apparent to me that the shorter exposures produce the same effect as the longer term effects?

7. Using toxicity data which are expressed as < or > incurs levels of uncertainty which may or may not be acceptable for such an exercise, however it is probably important to discuss that level of uncertainty.

8. The section on Treatment Process is most important for efficacy, however, it would probably be improved by presentation of some of the data that Page and team has collected on mixing, which might demonstrate the high variability of mixing in various treatments. There is some inference at the end of this paragraph that there is variance between label directions and fish treatment times which might be a problem, however, I would suggest this would seem to be minor and not worth identifying in an environmental effects review, unless there is a point I'm missing?

9. It seems that the cage edge method of calculations of toxicity is primarily directed at sessile organisms since it is those that are exposed to the approximate 1 hr plume passage If that is the case it should be stated. There are new exposures to water column animals (beyond those which were affected in the treatment containment) during that time, however the calculation of risk may be somewhat different since exposure concentrations are dropping rapidly in the water column. Should these exposures be separated?

10. The Net cage: plume coming from net cage section indicates (paragraph 8) a current speed value was selected for calculations (0.1 m s-1), without indicating whether that is a mean value or whether it is an upper end value. I believe these calculation should be done on upper end (25%?) representing a 'worse case' and certainly the variability in the real world situation should be presented.

11. The calculations indicate the dispersions occur uniformly and provide specific times and distances for dilution to non-toxic concentrations, which is appropriate for an overall risk calculation, however the level of patchiness and variability within the plume should probably be mentioned, again as a measure of the uncertainty in such calculations.

12. It might also be worth mentioning that as a plume disperses, while the concentration decreases, the time of exposure also increases, and the toxicity potential does not decrease in a strictly linear fashion. This is obviously not easy to model, and does argue for the value in obtaining real time toxicity measures in actual dispersing plumes.

13. The last paragraph in the Net Cage: plume coming from net cage section indicates that multiple treatment scenarios were not considered, because adequate data are not currently available, however is it possible, for the purposes of discussion to give some level of theoretical presentation. The single cage treatment is a very conservative risk situation and it would be worth giving some idea of the more realistic multiple treatment risk.

14. The Flushing Discharge Jet discussions become very non-quantitative and it is not clear whether that is a function of lack of data or lack of interpretation of data. Perhaps this could be clarified with some additional details.

15. The Summary and Conclusions indicates ratios (of what unstated?) were grouped into three categories, however the rationale, or the risk assessment precedence, for such categories is not indicated. Some additional discussion is suggested. It is notable that hazard quotients of 1 are usually the standard for screening level kinds of risk assessment decisions.

16. It is not clear to me why Table 16 does not present the absolute ratio values, as they could be quite informative from a comparative perspective.

17. I believe a summary table, similar to table 16 which presents the time and distance to non-toxic (distillation of Tables 4, 5a and 5b) is important to present and will give risk managers important decision-making information. What they particularly need to know is the zone of influence of these discharges and in a comparative way for products and methods of use would be most valuable.

18. I think the overall summary seriously underplays the risks. In my opinion you have demonstrated that using lethal effects information, impacts will occur beyond the end of pipe for Paramove®, Salmosan® and AlphaMax® in net pen and well boat use yet this is not mentioned? If the more conservative NOECs are used, which in my opinion is warranted, given the uncertainty of the analysis, there is a risk that toxic effects may occur much further afield.

19. Finally, since this is basically a risk assessment, I believe it may be appropriate to provide a list, with short description, of the uncertainties associated with the assessment.