Science

Sciences

## **Canadian Science Advisory Secretariat (CSAS)**

Research Document 2014/103
National Capital Region

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

F. H. Page and L. Burridge

Fisheries and Oceans Canada St. Andrews Biological Station 531 Brandy Cove Road St. Andrews, New Brunswick E5B 2L9



#### **Foreword**

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

## Published by:

Fisheries and Oceans Canada Canadian Science Advisory Secretariat 200 Kent Street Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/csas-sccs@dfo-mpo.gc.ca



© Her Majesty the Queen in Right of Canada, 2014 ISSN 1919-5044

## Correct citation for this publication:

Page, F.H., and Burridge, L. 2014. 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. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/103. v + 36 p.

# **TABLE OF CONTENTS**

ABSTRACT	IV
RÉSUMÉ	V
INTRODUCTION	1
THE TREATMENT PROCESS	2
TOXICITY OF THERAPEUTANTS	2
INSIDE THE TREATMENT VOLUME: TARPED OR WELL-BOAT	3
TOXICITY OF NET CAGE TREATMENTS ON NON-TARGET ORGANISMS	8
NET CAGE: CAGE EDGE OR END-OF-PIPE	8
NET CAGE: PLUME COMING FROM NET CAGE	8
TOXICITY OF WELL-BOAT TREATMENTS ON NON-TARGET ORGANISMS	14
WELL-BOAT: TOXICITY OF FLUSHING WATER DISCHARGE AT THE END-OF-PIPE	14
End-of-Pipe: Paramove <sup>®</sup> 50	
End-of-Pipe: Salmosan <sup>®</sup>	
End-of-Pipe: Alphamax <sup>®</sup>	22
WELL-BOAT: TOXICITY OF FLUSHING DISCHARGE JET	
Flushing Discharge Jet: Paramove <sup>®</sup> 50	30
Flushing Discharge Jet: Salmosan <sup>®</sup>	30
Flushing Discharge Jet: Alphamax <sup>®</sup>	30
SUMMARY	30
OVERALL CONCLUSIONS	35
ACKNOWLEDGEMENTS	36
REFERENCES	36

#### **ABSTRACT**

This paper represents an initial and preliminary effort to combine the transport and dispersal results of Page et al. (2014) with the toxicity results of Burridge (2013) in an effort to give a more field-oriented perspective on the spatial and temporal scales upon which therapeutant toxicity potentials may occur. The approach taken is to use a hazard quotient approach, i.e., the ratio of the estimated *in situ* exposure concentration to the level of effect concentration. The estimates of in situ therapeutant concentrations are estimated from the transport and dispersal relationships for tarp and well-boat bath treatments given in Page et al. (2014) and the organism-specific levels of effect concentrations are given by the LC<sub>50</sub> (the concentration at which 50% of test organisms die) and NOEC (the approximate concentration at which no lethal effect is observed) values reported in Burridge (2013). The results indicate that the horizontal spatial and temporal scales for potential toxic effects vary with the therapeutant, with the spatial and temporal scales increasing with the degree of therapeutant toxicity. The smallest scales (<100s of metres and minutes) are associated with Paramove®50 (active ingredient (a.i.) hydrogen peroxide) and the largest scales (kilometres and hours) are associated with Alphamax® (a.i. deltamethrin). The scales (100s of metres to a kilometre and minutes to hours associated with Salmosan® (a.i. azamethiphos) are intermediate to the other two therapeutants. It is also recognized that in order for these potentials to be realised, the *in situ* distributions of non-target organisms need to be such that they experience the above exposures.

Estimations des effets sur des organismes non ciblés des agents thérapeutiques chimiques contre le pou du poisson en provenance de déversements ou de rejets des bains thérapeutiques de cages en filets munis de bâches et de bateaux viviers : document de travail

### **RÉSUMÉ**

Ce document se veut un effort initial et préliminaire visant à combiner les résultats sur le transport et la dispersion de Page et al. (2014) aux résultats sur la toxicité de Burridge (2013) en vue d'obtenir un aperçu des échelles spatiales et temporelles auxquelles la toxicité potentielle des agents thérapeutiques peut survenir davantage centré sur la réalité du terrain. Cette approche consiste à utiliser une démarche fondée sur le quotient de risque (c.-à-d. le ratio de la concentration d'exposition in situ estimé par rapport au niveau de concentration à laquelle un effet est observé). Les estimations des concentrations d'agents thérapeutiques in situ sont estimées à partir des relations qui existent entre le transport et la dispersion des traitements donnés à l'aide de bains thérapeutiques munis de bâches et de bateaux viviers décrites dans Page et al. (2014), tandis que les niveaux de concentration à laquelle un effet est observé propres aux organismes sont établis en fonction de CL<sub>50</sub> (concentration à laquelle 50 % des organismes d'essai meurent) et de la concentration sans effet observé (CSEO) (concentration approximative à laquelle aucun effet létal n'est observé), des valeurs présentées dans Burridge (2013). Les résultats indiquent que les échelles spatiales et temporelles horizontales des effets toxiques possibles varient selon l'agent thérapeutique; les échelles spatiales et temporelles augmentant selon le degré de toxicité de l'agent. Les plus petites échelles (moins de 100 mètres et minutes) sont associées au Paramove®50 (matière active [m.a.] peroxyde d'hydrogène) et les plus grandes (kilomètres et heures) sont associées au Alphamax® (m.a. deltaméthrine). Les échelles (centaines de mètres à un kilomètre, et minutes à heures) associées au Salmosan® (m.a. azaméthiphos) sont intermédiaires entre les deux autres agents thérapeutiques. Il est également reconnu que pour que ces effets potentiels se réalisent, les organismes non ciblés doivent être répartis sur place de manière à être exposés selon les concentrations susmentionnées.

#### INTRODUCTION

Salmon farmers in southwest New Brunswick, and elsewhere in Canada and the world, need to control the abundance of sea lice on fish within their net-pens. There are several methods available for accomplishing this. One method is to administer pesticides in bath treatments. The potential environmental fate and effects of these treatments is the subject of this report.

In southwest New Brunswick bath treatments have been conducted in one of three ways: skirting, tarping, and well-boats. All of these are considered to be topical applications since the therapeutant is absorbed by the sea lice from the water. Skirt and tarp treatments involve reducing the depth of the net in the salmon cage, thus reducing the volume of water. The net and its enclosed salmon is either completely surrounded by an impervious tarpaulin (tarping) or a curtain or skirt is hung around the cage to a depth exceeding that of the enclosed salmon (skirting), and sufficient therapeutant is added to the enclosed volume to achieve the recommended treatment concentration. The salmon are maintained in the enclosed volume, or bath, for a period of time (usually 30 minutes) and aeration/oxygenation may be provided. After treatment, the tarpaulin or skirt is removed and the treatment chemical is allowed to disperse into the surrounding water.

Well-boat treatments are conducted by pumping salmon into wells or treatment chambers on specially designed vessels. Well-boats used in southwest New Brunswick typically have two wells, each capable of holding ~330 m³ of water. Fish are pumped into these wells, allowed to acclimate for a short period of time, and then pesticide is added to achieve a desired concentration. Mechanical aeration/oxygenation is provided. At the end of the prescribed treatment period (~30 minutes) the wells are flushed by simultaneously pumping out the treated water and replacing it with "clean" ambient seawater. Flushing periods are usually about twenty minutes in duration and after flushing is complete, the fish are pumped back into net-pens.

The potential of these chemical releases to affect non-target organisms is determined from estimates of exposure duration, intensity, and toxicity to the organisms. The exposure is determined by industrial discharge rates and processes and natural transport and dispersal processes. The potential effect on the non-target organisms is determined by the concentration and duration of the potential exposure coupled with the sensitivity of the organisms.

Since many factors influence the transport and discharge from tarped cages and well-boats (Page et al. 2014), the potential range of exposure concentrations and times likely to be experienced by *in situ* non-target organisms is large. These uncertainties are compounded with the assumptions and limitations of laboratory-based toxicity studies when assessing the uncertainties associated with the estimation of potential for harm to non-target organism.

In this report, the approach of calculating the ratio of the estimated *in situ* concentration to the laboratory estimated threshold for a particular effect is taken. The effects indicators used are the calculated concentration at which fifty percent of test organisms die, the  $LC_{50}$ , and the approximate concentration at which no lethal effect is observed, the NOEC. The *in situ* concentration of therapeutants is estimated for various times during the discharge sequence from commercial tarp and well-boat bath treatments. The estimates of *in situ* concentration are based on the relationships presented in Page et al. (2014) and the estimates of toxicity to non-target organisms are from the laboratory studies reported in Burridge (2013).

Predicting the fate of bath treatments in southwest New Brunswick has been attempted in the past. Burridge et al. (2000) employed a scaling analysis to predict the fate of Excis<sup>®</sup> (a.i. cypermethrin) from a treated net-pen. They concluded that the effluent would leave the cage in minutes and the potential for lethal consequences to non-target lobsters was small. They did,

however, caution about using their data to predict sublethal effects. Based on the recent work by Page et al. (2014), rapid dispersal of chemical from cages is not always observed. Ernst et al. (2001) used rhodamine dye to follow the effluent plume from a cage (no nets or fish). They concluded that there was potential for non-target effects especially with the use of Excis<sup>®</sup>. Crane et al. (2011) used published data on the lethality of deltamethrin to derive water quality guidelines for this therapeutant in Europe. No modeling of effluent was involved and these authors suggest that low ng/L concentrations can be considered safe for non-target exposure over a period of 3 h. Data presented in Burridge (2013) suggests that this level would not be protective of lobsters in southwest New Brunswick.

In the present report, an initial attempt is made to combine field and model-generated transport and dispersion estimates with laboratory estimated thresholds of lethality ( $LC_{50}$ ) and No Observable Effects Concentrations (NOEC) to determine length scales of potential impact. The analyses consider three sea lice therapeutants: Paramove®50 with the active ingredient hydrogen peroxide, Salmosan® with the active ingredient azamethiphos, and Alphamax® with the active ingredient deltamethrin. The thresholds are for American lobster larvae and adults, a mysid and Crangon. There is no attempt to explicitly consider the duration of the exposure, nor to consider multiple or pulsed exposures and cumulative effects.

The document is divided into four sections. The first section considers the toxicity of the therapeutants at their target concentrations. The second section considers the exposures and toxicities associated with net cage tarp treatments and the third section considers the exposures and toxicities associated with well-boat treatments. The final section summarizes the results.

The data and predictions presented here are based on exposure data and laboratory toxicity data analyses that were still in progress.

#### THE TREATMENT PROCESS

The concentration of therapeutant in the water inside a tarp or well varies with time. The concentration is initially zero, then the therapeutant is added and the concentration evolves spatially and temporally within the treatment volume until it becomes homogeneously mixed. Sufficient therapeutant is added that the well mixed concentration approximates the desired target treatment concentration ( $C_{treat}$ ). The time needed to fully mix the therapeutant varies with the cage size, tarping configuration, well-boat, and the rate of water recirculation within the tarp or well. In the case of tarping the flushing results from the natural flow of water through the cage. In the case of well-boats, the therapeutant is flushed from the well by mechanically pumping in ambient water and pumping out the water containing the therapeutant. The total length of time the therapeutant is retained within the tarp or well is therefore the time between the start of the rapeutant addition, or dosing, and the end of flushing. For industry operational purposes, the definition of treatment time is the time between the beginning of therapeutant dosing and the beginning of flushing. The consequence of this is that some fish may be treated for a longer period of time than is defined by the label. The flushing times for cage tarp treatments are much more variable than for well-boats. Flushing times in tarp treatments range from a few minutes to a few hours whereas in well-boats the industry typically has maintained flushing times of 15 to 25 minutes.

#### **TOXICITY OF THERAPEUTANTS**

Burridge (2013) presented data regarding the lethality of the three bath treatment products currently, or recently, applied to aquaculture cages in southwest New Brunswick. Lethality is reported as the  $LC_{50}$  or the concentrations lethal to 50% of the exposed organisms over a

prescribed time period (1 h or 24 h). NOECs are also presented in Burridge (2013) and used for calculations in the current analysis. The estimate of no effect was made by interpolation from lethality curves or as the mean of the lowest concentration with at least one mortality (or noted effect) and the highest concentration with no observed effects.  $LC_{50}$ s and NOECs presented in Burridge (2013) are sometimes reported as less than (<) or greater than (>) exposure values. In these tests, effects were observed at the lowest exposure concentration (<) or were not observed at the highest test concentration (>).

At each of the treatment stages, the potential toxicity of the therapeutant solution can be estimated by calculating the ratio of the therapeutant concentration ( $C_{th}$ ) to the concentration of toxic effects ( $C_{loe}$ ), i.e.,  $C_{th}/C_{loe}$ . When this ratio is greater than 1, the treatment or exposure concentration is greater than the effect concentration and the bath treatment is indicated as having potential to cause the specified effect. Conversely, when the ratio is less than 1, the treatment concentration is less than the effect concentration and the therapeutant bath is interpreted as being unlikely to cause the specified effect. The effect thresholds are taken from Burridge (2013). The authors recognize that there is uncertainty around these thresholds quantified as 95% confidence intervals reported in Burridge (2013). Since this document is a report on preliminary results and approaches, calculations are presented only for the threshold, not for the lower and upper confidence limits. Additionally, this simple approach is conservative in that it does not take into consideration the duration of the exposure. In other words, short exposures are assumed to illicit the same effect as long exposures.

The exposure concentrations calculated here are based on the dye studies reported by Page et al. (2014) and the assumption that there is a 1:1 relationship between the relative concentration of the therapeutants and the dye. Water samples were collected during the studies described by Page et al. (2014). The preliminary results of chemical analyses on these water samples are consistent with the assumption that dye concentration indicates the relative concentration of therapeutant. This finding is consistent with previous dye-therapeutant relationships obtained by Ernst et al. (2001). A more comprehensive examination of the relationship is planned for the ongoing work.

The threshold values of toxicity used here are from Burridge (2013), in which data for both lethal and sublethal effects of the three therapeutants on non-target organisms in laboratory studies were presented. The organisms selected include the American lobster, which is commercially important, and two other crustacean species that are found in the marine ecosystems of New Brunswick. These organisms have been used in studies because they are locally relevant, available and amenable to laboratory use, and are expected to be sensitive to these classes of chemicals. The threshold values used in this paper represent the range of sensitivity of organisms as presented in Burridge (2013) and include the most sensitive endpoints identified for marine organisms.

### INSIDE THE TREATMENT VOLUME: TARPED OR WELL-BOAT

The toxicity of the treatment concentrations to non-target organisms is examined below since it is a common practice of some regulatory authorities to take an end-of-pipe perspective. Hence, for tarp or skirt net-pen treatments, the end-of-pipe corresponds to the outside edge of the net-pen. For well-boats, the end-of-pipe concentration is usually a diluted version of the treatment concentration due to mechanical flushing processes (see later section). However, if flushing is not implemented, the end-of-pipe could be the treatment concentration. For tarp treatments, it is assumed that the treatment concentration has been reached and is well mixed within the tarp at release.

As indicated above, estimates of the potential toxicity of treatment target concentrations within treatment containers have been calculated as  $C_{treat}/C_{loe}$  for the three examined therapeutants, the three test non-target organisms, the derived LC<sub>50</sub> and NOEC thresholds and the different treatment approaches.

The potential toxicity ratios for Paramove<sup>®</sup>50 treatments using target concentrations of 1200 mg of active ingredient (a.i.) hydrogen peroxide L<sup>-1</sup> are presented in Table 1. The LC<sub>50</sub> based ratios indicate that adult and larval lobsters as well as Crangon enclosed in the Paramove<sup>®</sup>50 bath treatments are unlikely to experience mortality, and mysids are likely to experience significant mortality. The NOEC based ratios indicate that adult lobsters are less likely to experience observable effects compared to lobster larvae, mysids and Crangon, which are likely to experience significant observable effects.

Table 1: The ratio ( $\overline{C}_0/C_{loe}$ ) of the estimated target concentration ( $\overline{C}_0$ ) of Paramove<sup>®</sup>50, expressed as hydrogen peroxide, to the level of effect values ( $C_{loe}$ ) for various organisms. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of hydrogen peroxide and organism exposures of 1 h. The treatment concentration of hydrogen peroxide is assumed to be 1200 mg/L. Shaded cells highlight ratio values that are greater than one and indicate a potential for toxic effects.

	I	I			
Test Organism	Level of Effect	Ratio ( C̄₀/C <sub>loe</sub> )			
	C <sub>loe</sub> = 1-h LC <sub>50</sub> lethal (mg/L)				
Lobster Larvae Stage I	1637	0.7			
Lobster Adults	>3750	<0.3			
Mysids	973	1.2			
Crangon	3182	0.4			
	C <sub>loe</sub> =1-h NOEC lethal (mg/L)				
Lobster Larvae Stage I	356	3.4			
Lobster Adults	971	1.2			
Mysids	<245 >4.9				
Crangon	<223	>5.4			

The ratios for Salmosan® treatments using target concentrations of 100 µg of active ingredient azamethiphos L-1 are presented in Table 2. The LC50 based ratios indicate that larval lobsters, mysids and Crangon enclosed in the Salmosan® bath treatments may or may not all experience mortality, but that significant adult lobster mortality could occur. The NOEC based ratios indicate that lobster larvae, adult lobsters, mysids and Crangon are all likely to experience significant effects.

Table 2: The ratio ( $\overline{C}_0/C_{loe}$ ) of the estimated target concentration ( $\overline{C}_0$ ) of Salmosan<sup>®</sup>, expressed as azamethiphos, to the level of effect values ( $C_{loe}$ ) for various organisms. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of azamethiphos and organism exposures of 1 h and 10 d. The treatment concentration is assumed to be 100 µg/L a.i. azamethiphos. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects. The 1-h NOEC lethal are based on observed lowest exposure concentration where no death occurred.

Test Organism	Level of Effect	Ratio ( Ū₀/C <sub>loe</sub> )			
	C <sub>loe</sub> = 1-	-h LC <sub>50</sub> lethal (μg/L)			
Lobster Larvae Stage I	>86.5 <1.2				
Lobster Adults	24.8	4.0			
Mysids	>85.5	<1.2			
Crangon	>85.5	<1.2			
	C <sub>loe</sub> = 10-d LC <sub>50</sub> lethal (µg/L)				
Lobster Adults	0.216	463.0			
	C <sub>loe</sub> = 1-l	h NOEC lethal (μg/L)			
Lobster Larvae Stage I	<0.37	>270.3			
Lobster Adults	9.85	10.2			
Mysids	<0.97	>103.1			
Crangon	<0.97	>103.1			
	C <sub>loe</sub> = 10-d NOEC lethal (μg/L)				
Lobster Adults	0.123	813.0			

Table 3: The ratio ( $\overline{C}_0/C_{loe}$ ) of the estimated target concentration ( $\overline{C}_0$ ) of Alphamax $^{\otimes}$ , expressed as deltamethrin, to the LC<sub>50</sub> level of effect values ( $C_{loe}$ ) for various organisms. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h and on measured concentrations for 24 h and 10 d. The treatment concentration of deltamethrin is assumed to be 2000 ng/L. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Test Organism	Level of Effect	Ratio ( C̄₀/C <sub>loe</sub> )			
	$C_{loe}$ = 1-h LC <sub>50</sub> lethal (ng/L)				
Lobster Larvae Stage I	3.4	588.2			
Lobster Larvae Stage III*	36.5	54.8			
Lobster Adults	18.8	106.4			
Mysids	13.9	143.9			
Crangon**	142	14.1			
	C <sub>loe</sub> = 24-h LC	c <sub>50</sub> lethal (ng/L)			
Lobster Larvae Stage I	0.8	2500.0			
Lobster Larvae Stage II	0.6	3333.3			
Lobster Larvae Stage IV	1.7	1176.5			
Lobster Adults	15.0	133.3			
Mysids	1.4	1428.6			
Crangon	27.0	74.1			
	C <sub>loe</sub> = 10-d LC <sub>50</sub> lethal (ng/L)				
Lobster Adults	14.7	136.1			

<sup>\*</sup> based on nominal effects concentrations 1 h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

<sup>\*\*</sup> nominal effects concentrations from Fairchild et al. (2010)

Table 4: The ratio  $(\overline{C}_0/C_{loe})$  of the estimated target concentration  $(\overline{C}_0)$  of Alphamax<sup>®</sup>, expressed as deltamethrin, to the NOEC effect values  $(C_{loe})$  for various organisms. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, and on measured concentrations for 24 h and 10 d. The treatment concentration deltamethrin is assumed to be 2000 ng/L. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Test Organism	Level of Effect	Ratio (C <sub>0</sub> /C <sub>loe</sub> )			
	C <sub>loe</sub> = 1-h NOE	EC lethal (ng/L)			
Lobster Larvae Stage I	<0.6	>3333.3			
Lobster Adults	3.6	555.5			
Mysids	0.9	2222.2			
	C <sub>loe</sub> = 24-h NO	EC lethal (ng/L)			
Lobster Larvae Stage I	<0.08	>25000.0			
Lobster Larvae Stage II	<0.08	>25000.0			
Lobster Larvae Stage IV	<0.08	>25000.0			
Lobster Adults	4.8	416.7			
Mysids	<0.2	>10000.0			
Crangon	5	400.0			
	C <sub>loe</sub> = 24-h NOE0	C sublethal (ng/L)			
Lobster Larvae Stage I	<0.08	>25000.0			
Lobster Larvae Stage III	<0.08	>25000.0			
Lobster Larvae Stage IV	<0.08	>25000.0			
Lobster Adults	<0.6	>3333.3			
Mysids	<0.2	>10000.0			
Crangon	<8	>250.0			
	C <sub>loe</sub> = 10-d NOEC lethal (ng/L)				
Lobster Adults	5	400.0			

#### TOXICITY OF NET CAGE TREATMENTS ON NON-TARGET ORGANISMS

### **NET CAGE: CAGE EDGE OR END-OF-PIPE**

To a first approximation, the toxicity of the therapeutants at the edge of the net cage is the same as that inside the tarp or skirted cage. A refinement of this approximation could take into consideration the duration of the exposure. As explained in Page et al. (2014), the therapeutant may leave the cage as a plug flow or be leaked out over time. In the former situation the duration of exposure can be estimated as the time needed for the therapeutant to traverse the diameter of the cage (d). This is estimated as  $t_{fl.cage} = d/u_{cage}$ , where  $u_{cage}$  is the speed of the water moving through the cage. Unfortunately, this is seldom well known and making the assumption that it is the same as the ambient current speed usually overestimates the speed and hence would underestimate the flushing time or exposure duration. Despite this uncertainty, experience suggests the flushing times are of order minutes to an hour or more (Page et al. 2014). These exposures are consistent with the 1-h exposure experiments conducted by Burridge (2013) and the estimated toxicity of the therapeutant to non-target organisms located near the edge of the cage would be the same as those given above for the treatment concentrations. If the therapeutant concentration in the cage decreases over time in an exponential manner, the mean concentration seen by non-target organisms nearby may be approximated by

$$\overline{C}_{eop} = \frac{C_0}{\alpha} \left[ 1 - \exp\left(-\alpha t_{\text{max}}\right) \right]$$

where  $\alpha$  is the estimated rate of decrease in the therapeutant concentration. If  $\alpha t_{max}$  is assumed to equal to -3, i.e., 95% of the therapeutant has left the cage, then the average concentration experienced by organisms near the cage edge is 0.95  $C_0/3$ =0.32 $C_0$ . Although this reduces the ratios presented in Tables 1-4 it does not substantially change the conclusions, hence the reduced ratios have not been presented here.

### **NET CAGE: PLUME COMING FROM NET CAGE**

To a first approximation, the temporal evolution of the spatial average concentration of therapeutant within the plume resulting from a net cage treatment can be estimated by the relationship given in Page et al. (2014)

$$\overline{C}(t_0 + \Delta t) = \frac{M}{V_t} = \frac{M}{A_t h_t} = \frac{M}{\pi r^2 h_t} = \frac{4M}{9\pi \cdot 2.5 \cdot 10^{-9} (t_0 + \Delta t)^3 h_t}$$

In this relationship the average concentration ( $\bar{C}$  in Mass per m³) of the therapeutant patch is estimated as the mass (M) of therapeutant used in the treatment divided by the temporally evolving volume of the patch ( $V_t$  in m³) estimated as the area of the patch ( $A_t$  in m²) times the depth of the layer ( $h_t$  in m) over which the therapeutant is mixed. The area is defined as ( $A_t = \pi r_t^2 = \pi d_t^2/4$ ) in which the radius ( $r_t$ ) and diameter ( $d_t$ ) are evolving over time (t in seconds). When the diameter (d) of the patch is replaced by the Okubo length scale ( $3\sigma_r$ ) the area of the patch becomes  $A = \pi (3\sigma_r)^2/4 = 9\pi\sigma_r^2/4$  and the volume of the patch is given by  $V_t = \pi r^2 h_t = 9\pi\sigma_r^2 h_t/4$ . Furthermore, when the Okubo variance is replaced by  $\sigma_{r(t)}^2 = 2.5 \cdot 10^{-9} (t_0 + \Delta t)^3$ , the volume at any given time ( $t_0 + \Delta t$ ) is given by  $V_t = 9\pi 2.5 \cdot 10^{-9} (t_0 + \Delta t)^3 h_t/4$  in which  $t_0$  is the time at which the Okubo length scale equals the diameter of the cage and  $\Delta t$  is the time since the dropping of the tarp. At the time of release,  $\Delta t = 0$  and the average concentration equals the target concentration. The Okubo (1974) relationship describing the rate at which a dispersing patch increases in size with time seems to provide a reasonable, although perhaps

underestimate, of the size of dispersing dye patches released from fish cages (Page et al. Unpublished Manuscript<sup>1</sup>, Page et al. 2014). This means the average concentrations estimated by the above equation may be biased upward.

The time needed to dilute the initial concentration to the level of effects threshold ( $C_{loe}$ ) can be estimated as

$$\Delta t_{loe} = \sqrt[3]{\frac{4M}{9\pi 2.5 \cdot 10^{-9} h C_{loe}}} - t_0$$

where  $\Delta t_{loe}$  is in seconds when h is in metres and  $C_{loe}$  is in units of mass per cubic metre.

The area occupied by the patch at the time the average concentration reaches the level of effect can be estimated as

$$A_{loe} = \frac{9\pi \ 2.5 \cdot 10^{-9} \ (t_0 + \Delta t_{loe})^3}{4}$$

in which

$$A_{loe} = \pi r_{loe}^2 = \pi (d_{loe}/2)^2 = \pi (3\sigma_{loe}/2)^2 = (\pi 9\sigma_{loe}^2)/4$$

and  $r_{loe}$  and  $d_{loe}$  are the radius and diameter of the Okubo patch at the time when  $C_t = C_{loe}$ .

If we further assume that the patch is actually an ellipse rather than a circle, and that the major axis of the ellipse is three times longer than the minor axis, the length of the major axis ( $I_x$ ) at the time when  $C_t = C_{loe}$  can be estimated as

$$l_x = 2\sqrt{3A_{loe}/\pi}$$

Although this is a crude assumption, it is consistent with the observations shown in Page et al. (2014).

The distance of the therapeutant patch center  $(d_{pc})$  from the release location, assuming an instantaneously release, depends upon the mean speed of the water current at the time of release, i.e.,  $d_{pc} = \bar{u}\Delta t$ . The distance of the leading edge of the patch from the treatment cage is approximated by  $d_{ple} = u\Delta t + I_x/2$ .

Tables 5 and 6 show values calculated using the above equations for Salmosan® and Alphamax® treatments conducted in a 100 m perimeter net-pen. Calculations were not made for Paramove®50 since at this time it is not used in tarp treatments in southwest New Brunswick. The analyses assume that the concentration within the patch of therapeutant is uniformly distributed and that the toxicity potential within the plume decreases according to Okubo-based estimate of the reduction in pesticide concentration. The assumption of a uniform distribution is a simplification since observed concentration within a dispersing plume tends not to be uniform (Page et al. 2014). Also, unless otherwise indicated, all of the calculations shown below assumed an advective velocity of u = 0.1 m/s, an intermediate value observed during the release studies (Page et al. 2014) and a value of h = 5 m for the thickness or maximum depth of the therapeutant layer. Since the thickness of the therapeutant layer is sometimes greater than

<sup>-</sup>

<sup>&</sup>lt;sup>1</sup> Page, F.H., Chang, B.D., and Losier, R. 1998 (unpublished). Relative dispessal of Rhodamine WT Dye and Cypermethrin from salmon sea pens located within the Quoddy Region of southwestern New Brunswick; some preliminary analyses. An internal report to Environment Canada. 19 p.

this, the calculated dilution time and length scales tend to represent the larger of the values that are likely to be realized *in situ*. It should also be noted that values are presented for 10-day  $LC_{50}$  lethal and NOEC levels of effect. However, these values must be interpreted carefully, since it is unlikely that in normal circumstances *in situ* organisms will experience the reported  $LC_{50}$  concentrations continuously for 10 d.

Table 5: Dilution times and zone of effect estimates for Salmosan<sup>®</sup>, expressed as azamethiphos, for various organisms. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of azamethiphos and organism exposures of 1 h and 10 d. The treatment concentration is assumed to be 100  $\mu$ g/L a.i. azamethiphos and the patch is assumed to remain in the upper 5 m of the water column. The calculations are based on the Okubo relationship.

Test Organism	Level of Effect	Time (min)	Plume Area (m²)	Length of Plume major Axis (m)	Distance of Patch Center from cage u=0.1m/s (m)	Leading edge distance (m)	
			C <sub>loe</sub> = 1-h L	.C <sub>50</sub> lethal	(µg/L)		
Lobster Larvae Stage I	>86.5	0	796	32	0	CE	
Lobster Adults	24.8	28	2567	99	170	219	
Mysids	>85.5	0	796	32	0	CE	
Crangon	>85.5	0	796	32	0	CE	
		(	C <sub>loe</sub> = 10-d	LC <sub>50</sub> lethal	(µg/L)		
Lobster Adults	0.240	352	265,258	1,007	2,111	2,614	
		(	C <sub>loe</sub> = 1-h No	OEC letha	l (μg/L)		
Lobster Larvae Stage I	<0.37	297	172,059	811	1,780	2,185	
Lobster Adults	9.85	60	6463	157	359	438	
Mysids	0.97	199	65,631	501	1,193	1,443	
Crangon	0.97	199	65,631	501	1,193	1,443	
	C <sub>loe</sub> = 10-d NOEC lethal (μg/L)						
Lobster Adults	0.123	454	517,577	1,406	2,727	3,430	

For Salmosan® the 1-h LC<sub>50</sub>'s for all test organisms, except adult lobsters, were greater than the treatment concentration, hence for most of the test organisms the time required to reach the level of effect is zero (which means there is no effect and the scales of the plume are the size of

the treatment cage). Adult lobsters were, however, affected by the Salmosan $^{\$}$ . It took about 28 minutes to dilute the treatment concentration to an average concentration equal to the 1-h LC $_{50}$  level of effect. During this time the length of the dispersing plume is approximately 99 m, and the distance the patch travels away from the treated cage is 170 m and the leading edge distance is 219 m. The area of the patch when the concentration equals the threshold concentration is about a thousand square meters.

When the more conservative or protective 1-h NOEC levels of effect are considered, the time needed to dilute the Salmosan® treatment concentration increased substantially to a few hours, the patch length increased to a few hundreds of meters and the distance of influence increases to hundreds of meters to over a kilometer. The area of the patch when the concentration equals the threshold concentration is thousands of square metres to over a hundred thousand square meters.

The treatment concentration of Alphamax® (2000 ng/L = 2  $\mu$ g/L) is considerably lower than for Salmonsan® (100  $\mu$ g/L), it is also more toxic to the test organisms, and hence the time required to dilute Alphamax® to the level of effect is longer than for Salmosan® (Tables 6 and 7). The size of the estimated discharge plume once the average plume concentration equaled the level of effect concentration, and the estimated distance that the plume will travel away from the treated cage is also greater for Alphamax® than for Salmosan®.

The times to dilute to the 1-h  $LC_{50}$ 's range from 1 to 13 hours with the time being longest for stage II lobster larvae. Crangon were the least sensitive species. During the dilution times the length of the dispersing plume ranged from 185 m to 2.8 km and the distance the patch travelled away from the treated cage ranged from 442 m to 4.6 km. The leading edge of the patch at the time when the dilution equals the level of effect ranges from 534 m to 3.0 km.

When the more conservative 1-h NOEC levels of effect are considered, the time needed to dilute the Alphamax<sup>®</sup> treatment concentration were even longer (Table 6), and ranged from 5-26 hours. The patch length scales range from about 800 m to 8 km and the distance of influence ranges from 2 to 13 km.

All of the above pertains to single, isolated therapeutant treatments. In practice the industry conducts multiple treatments per day per farm and on multiple farms per day. The combination of these multiple treatments coupled with varying directions of transport due to the tidal variation in the currents will generate larger cumulative zones of potential impact than indicated by the above. These multiple release scenarios are not discussed here but they are the topic of further and ongoing research.

Table 6: Dilution times and zone of effect estimates for Alphamax<sup>®</sup>, expressed as deltamethrin, for various organisms. The  $C_{loe}$  are  $LC_{50}$  values from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h and on measured concentrations for 24 h and 10 d. The treatment concentration is assumed to be 2000 ng/L a.i. deltamethrin and the patch is assumed to remain in the upper 5 m of the water column. The calculations are based on the Okubo relationship.

Test Organism	Level of Effect	Time (h)	Plume Area (m²)	Length of Plume major Axis (m)	Distance of Patch Center from cage u=0.1m/s (m)	Leading edge distance (m)	
			C <sub>loe</sub> = 1-h l	_C <sub>50</sub> lethal	(ng/L)		
Lobster Larvae Stage I	3.4	6.70	374482	1196	2412	3010	
Lobster Larvae Stage III*	36.5	2.50	34883	365	899	1081	
Lobster Adults	18.8	3.36	67726	509	1209	1463	
Mysids	13.9	3.82	91600	592	1375	1671	
Crangon**	142.0	1.23	8966	185	442	534	
			C <sub>loe</sub> = 24-h	LC <sub>50</sub> letha	I (ng/L)		
Lobster Larvae Stage I	0.8	11.46	1591549	2466	4127	5359	
Lobster Larvae Stage II	0.6	12.72	2122066	2847	4578	6001	
Lobster Larvae Stage IV	1.7	8.70	748964	1691	3131	3976	
Lobster Adults	15.0	3.70	84883	569	1331	1616	
Mysids	1.4	9.34	909457	1864	3364	4296	
Crangon	27.0	2.86	47157	424	1031	1243	
	$C_{loe}$ = 10-d LC <sub>50</sub> lethal (ng/L)						
Lobster Adults	14.7	3.73	86615	575	1343	1630	

<sup>\*</sup> based on nominal effects concentrations 1 h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

<sup>\*\*</sup> nominal effects concentrations from Fairchild et al. (2010)

Table 7: Dilution times and zone of effect estimates for Alphamax<sup>®</sup>, expressed as deltamethrin, for various organisms. The  $C_{loe}$  are NOEC values from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h and on measured concentrations for 24 h and 10 d. The treatment concentration is assumed to be 2000 ng/L a.i. deltamethrin and the patch is assumed to remain in the upper 5 m of the water column. The calculations are based on the Okubo relationship.

	1 1		T	1		ı		
Test Organism	Level of Effect	Time (h)	Plume Area (m²)	Length of Plume major Axis (m)	Distance of Patch Center from cage u=0.1m/s (m)	Leading edge distance (m)		
			C <sub>loe</sub> = 1-h l	NOEC letha	al (ng/L)			
Lobster Larvae Stage I	0.6	12.72	2122066	2847	4578	6001		
Lobster Adults	3.6	6.55	353678	1162	2359	2940		
Mysids	0.9	11.0	1414711	2325	3954	5116		
			$C_{loe} = 24-h$	NOEC leth	al (ng/L)			
Lobster Larvae Stage I	0.08	25.84	15915494	7797	9301	13200		
Lobster Larvae Stage II	0.08	25.84	15915494	7797	9301	13200		
Lobster Larvae Stage IV	0.08	25.84	15915494	7797	9301	13200		
Lobster Adults	4.8	5.86	265258	1007	2111	2614		
Mysids	0.2	18.78	6366198	4931	6760	9225		
Crangon	5	5.77	254648	986	2078	2571		
			$C_{loe} = 24-h N$	OEC suble	thal (ng/L)			
Lobster Larvae Stage I	0.08	25.84	15915494	7797	9301	13200		
Lobster Larvae Stage II	0.08	25.84	15915494	7797	9301	13200		
Lobster Larvae Stage IV	0.08	25.84	15915494	7797	9301	13200		
Lobster Adults	0.6	12.72	2122066	2847	4578	6001		
Mysids	0.2	18.78	6366198	4931	6760	9225		
Crangon	8	4.79	159155	780	1725	2115		
		C <sub>loe</sub> = 10-d NOEC lethal (ng/L)						
Lobster Adults	5	5.77	254648	986	2078	2571		

#### TOXICITY OF WELL-BOAT TREATMENTS ON NON-TARGET ORGANISMS

The target therapeutant concentration used in well-boats is the same as described in the "Inside the treatment volume" section. The treatment water from a well-boat treatment is mechanically pumped from the vessel into the ambient receiving environment. The discharge therefore initially takes the form of a jet flow that evolves into a plume that is transported and dispersed by the ambient advection and eddy diffusion processes.

This section of the report considers the estimated toxicity of the end-of-pipe discharge when mechanical flushing was initiated, as well as the toxicity within the discharge jet. The toxicities within the plume evolving from the discharge jet are beyond the scope of this report and hence are not evaluated. The potential toxicity of three anti sea lice therapeutants: Paramove®50, Salmosan®, and Alphamax®, are evaluated. If the treatment water is considered non-toxic to the non-target organisms, then the waters flushed out of the well are therefore not likely to be toxic. Similarly, if the end-of-pipe waters are not toxic, then the therapeutants in the discharge jet and plume are not likely to be toxic. Finally, if the therapeutants in the discharge jet are not toxic, then the therapeutants in the discharge plume are not likely to be toxic.

## WELL-BOAT: TOXICITY OF FLUSHING WATER DISCHARGE AT THE END-OF-PIPE

The process of flushing treated water from the well-boat wells results in a dilution of the therapeutant over time (Page et al. 2014) and this dilution may reduce the concentration of therapeutant to below levels of regulatory concern. In order to determine the potential of this dilution effect, the concentration at the end of the flushing discharge pipe and/or the average end-of-pipe concentration during the flushing time period can be compared to  $LC_{50}$  and NOEC values of non-target organisms. To do this, the water within the well is assumed to rapidly mix and the concentration of therapeutant in the flushing discharge flow decreases exponentially with time. Under these circumstances the therapeutant within the treatment well and at the end of the discharge pipe ( $C_{eop}$ ) decreases exponentially with time and is represented reasonably well by the relationship (Page et al. 2014)

$$C_{eop}(t) = C_0 \exp(-Qt/V)$$

where  $C_0$  is the homogeneous concentration of therapeutant in the well just prior to the commencement of flushing, Q is the volume flow of water pumped from the well during flushing and V is the volume of the well.  $C_0$  is taken as the target therapeutant concentration or can be calculated as M / V where M is the total mass of therapeutant added to the well and V is the volume of water within the well.

The concentration of therapeutant exiting the end-of-pipe at the end of a specified flushing period can be estimated from the above equation by substituting t with  $t_{fl}$ , the duration of an operational flushing time, so

$$C_{eop}(t) = C_0 \exp(-Qt_f / V).$$

The average concentration over the specified flushing period can be estimated as

$$\overline{C}_{eop} = \frac{\int_0^{t_f} C_0 \exp\left(-Qt/V\right) \partial t}{t_f} = \frac{C_0 V}{Qt_f} \left[1 - \exp\left(-Qt_f/V\right)\right]$$

and the flushing times needed to reduce  $C_{\text{max}}$  to a specific LC<sub>50</sub> or NOEC values can be estimated as

$$t_{LC50} = -Ln (LC_{50} / C_0) \cdot (V / Q)$$

and

$$t_{NOEC} = -Ln (NOEC / C_0) \bullet (V / Q).$$

In order to calculate the various ratios indicating the potential for toxicity, values for pumping rates (Q), well volumes (V), flushing times ( $t_{fl}$ ), treatment concentrations ( $C_0$ ), LC<sub>50</sub>'s and NOEC's are needed. Fortunately, reasonable estimates for all of these parameters exist, except for pumping rate. Typical commercial flushing times are 15-30 minutes; a value of 20 minutes is assumed in the calculations below. The pumping rates of the well-boats in southwest New Brunswick are not well known. However, based on the author's discussions with well-boat Captains and engineers, it is suggested that the pumps in the well-boats used in New Brunswick are rated for a maximum of between 3500 and 3000 m³/h (F. Page, Unpublished Manuscript¹). These discussions also indicate that the pumps are not run at full capacity, and dye work conducted (Page et al. 2014) suggests the pumping rates vary between treatments and may at times be as low as 20-30% of the maximums. Therefore, in the examples presented below, a range of pumping rates was chosen to reflect the range of pumping rates that might be experienced in the fleet of well-boats operating in the southwest New Brunswick area during 2010-11. Pumping rates between 1200 and 2400 m³/h are probably the most representative, based on current information. Efforts are being made to get better estimates of the pumping rates.

# End-of-Pipe: Paramove<sup>®</sup>50

The ratios of the concentration of hydrogen peroxide at the end of the discharge pipe, averaged over the flushing duration, and the concentration at the end of the discharge pipe at the end of the 20 minute flushing period, in relation to the laboratory derived concentration levels of effect, are presented in Tables 8 and 9. In both tables the end-of-pipe concentrations are based on a range of well pumping rates and a hydrogen peroxide target bath concentration of 1200 mg/L ( $\equiv$ ppm). The effects concentrations used are LC<sub>50</sub> and NOEC lethal values for Paramove<sup>®</sup>50, with active ingredient hydrogen peroxide. The effects thresholds are from Burridge (2013) and are derived from 1-h continuous exposure laboratory experiments.

Table 8: The ratio( $\overline{C}_{eop}/C_{loe}$ ) of the estimated average concentration ( $\overline{C}_{eop}$ ) of Paramove<sup>®</sup>50 during flushing, expressed as hydrogen peroxide, at the end of the flushing pipe to the level of effect values ( $C_{loe}$ ) for various organisms and pumping rates. The  $C_{effect}$  values are from Burridge (2013) and are based on measured concentrations of hydrogen peroxide and organism exposures of 1 h. The well volume is assumed to be 330 m³ and the treatment concentration of hydrogen peroxide is assumed to be 1200 mg/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect		Ratio ( $\bar{C}_{eop}/C_{loe}$ )							
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)			
		$C_{loe}$ = 1-h LC <sub>50</sub> lethal (mg/L)								
Lobster I	1637	0.214	0.251	0.301	0.369	0.464	0.600			
Lobster Adults	>3750	0.086	0.100	0.120	0.147	0.185	0.240			
Mysids	973	0.330	0.387	0.508	0.464	0.715	0.925			
Crangon	3182	0.101	0.118	0.142	0.174	0.219	0.283			
		C	C <sub>loe</sub> = 1-h <b>l</b>	NOEC let	hal (mg/L	.)				
Lobster I	356	0.903	1.059	1.267	1.553	1.953	2.528			
Lobster Adults	971	0.331	0.388	0.465	0.569	0.716	0.927			
Mysids	<245	1.311	1.538	1.842	2.257	2.838	3.673			
Crangon	<223	1.441	1.690	2.023	2.479	3.118	4.036			

Table 9: The ratio ( $C_{eop}$  ( $t = t_{fl}$ ) /  $C_{effect}$ ) of the estimated concentration of Paramove  $^{®}$ 50, expressed as hydrogen peroxide, at the end of the flushing discharge pipe at the end of the flushing period ( $C_{eop}$  ( $t = t_{fl}$ )) to the  $C_{effect}$  values for various organisms and pumping rates. The  $C_{effect}$  values are from Burridge (2013) and are based on measured concentrations of hydrogen peroxide and organism exposures of 1 h. The well volume is assumed to be 330 m³ and the treatment concentration of hydrogen peroxide is assumed to be 1200 mg/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect	Ratio $(C_{eop}(t = t_{fl}) / C_{loe}))$							
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)		
		$C_{loe}$ = 1-h LC <sub>50</sub> lethal (mg/L)							
Lobster I	1637	0.019	0.035	0.065	0.119	0.218	0.400		
Lobster Adults	>3750	0.008	0.015	0.028	0.052	0.095	0.175		
Mysids	973	0.032	0.060	0.109	0.200	0.367	0.673		
Crangon	3182	0.010	0.018	0.033	0.061	0.112	0.206		
		$C_{loc}$	<sub>e</sub> = 1-h NC	EC letha	ıl (mg/L)				
Lobster I	356	0.089	0.163	0.298	0.547	1.003	1.839		
Lobster Adults	971	0.033	0.060	0.109	0.201	0.368	0.674		
Mysids	<245	0.129	0.237	0.434	0.795	1.457	2.676		
Crangon	<223	0.142	0.260	0.476	0.873	1.601	2.935		

All of the time averaged ratios ( $\overline{C}_{eop}/LC_{50}$ ) indicate that the end-of-pipe concentrations are much less than the  $LC_{50}$  values (Table 8). This indicates that the Paramove<sup>®</sup>50 flushed from the wells is not toxic at the LC<sub>50</sub> levels to the ambient waters and that the concentration of hydrogen peroxide in the water remaining in the well after flushing is less than the LC<sub>50</sub> lethal effects concentrations.

With the exception of adult lobsters and the 3600 m<sup>3</sup>/h pumping rate for stage I lobster larvae, all of the  $\bar{C}_{eop}$ /NOEC ratio values are greater than one (Table 8), indicating that the end-of-pipe concentrations are greater than the NOEC values reported by Burridge (2013) and hence have toxic potential. The ratios for adult lobsters are all less than one, indicating the concentration of hydrogen peroxide remaining in the well after flushing was less than the NOEC lethal of effects concentration for adult lobsters.

With the exception of the 600-1200 m<sup>3</sup>/h pumping rates for mysids, Crangon, stage I lobster larvae for 1-h NOEC levels of effect, all of the  $C_{eop}$  ( $t = t_{fl}$ ) /  $C_{loe}$  ratio values involving 1-h LC<sub>50</sub> and NOEC levels of effect, are also less than one (Table 9). This indicates that the end-of-pipe

concentrations at the end of flushing are less than the 1-h  $LC_{50}$  and NOEC values reported by Burridge (2013) and that discharged water at this time, is for the most part not toxic to lobsters, mysids or Crangon shrimp. The ratio values for mysids, Crangon and stage I lobster larvae indicate that at the slower pumping rates the concentration of hydrogen peroxide in the water remaining in the well after flushing is greater than the NOEC lethal effects concentrations and hence some end-of-pipe toxicity potential exists.

The flushing times needed to reduce the end-of-pipe concentration to the Burridge (2013)  $LC_{50}$  lethal values ( $t_{LC50}$ ) for the above Paramove<sup>®</sup>50 treatments are presented in Table 10. With the exception of the mysids, all of the  $t_{LC50}$  values were negative, indicating that the effects levels were greater than the treatment concentrations and that flushing does not need to occur to reduce the concentrations below the effects levels. For mysids, the treatment concentrations were greater than the effects concentrations and the times to reduce the treatment concentrations were less that the operational flushing times.

Table 10: Duration of flushing needed for end-of-flushing-pipe concentration for Paramove  $^{\otimes}$ 50, expressed as hydrogen peroxide, to be reduced to  $LC_{50}$  ( $t_{LC50}$ ) or NOEC values for various organisms and pumping rates. The level of effect values are from Burridge (2013) and are based on measured concentrations of hydrogen peroxide and organism exposures of 1 h. The well volume (V) is assumed to be 330 m³ and the treatment concentration of hydrogen peroxide is assumed to be 1200 mg/L. Negative values indicate the treatment concentration is less than the  $LC_{50}$  effects concentration. Typical operational flushing times are 20 minutes.

Organism	Level of Effect	Time (min) to Levels of Effect								
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)			
		1-h LC <sub>50</sub> lethal (mg/L)								
Lobster I	1637	-2	-2	-3	-3	-5	-10			
Lobster Adults	>3750	-6	-8	-9	-13	-19	-38			
Mysids	973	1	1	2	1	3	7			
Crangon	3182	-5	-6	-8	-11	-16	-32			
			1-h NO	EC lethal	(mg/L)					
Lobster I	356	7	8	10	13	20	40			
Lobster Adults	971	1	1	2	2	3	7			
Mysids	<245	9	10	13	17	26	52			
Crangon	<223	9	11	14	19	28	56			

Table 10 also shows the flushing times ( $t_{NOEC}$ ) needed to reduce the end-of-pipe concentration to the NOEC lethal values for Paramove<sup>®</sup> 50 treatments. All of the  $t_{NOEC}$  values were positive and those at the slower pumping rates could be longer than typical operational flushing times. At the slow pumping rates the flushing times would need to be tripled to reduce the concentration of hydrogen peroxide at the end of the discharge pipe to levels below the NOEC.

## End-of-Pipe: Salmosan®

The ratios of the concentration of azamethiphos at the end of the discharge pipe averaged over the flushing duration and the concentration at the end of the discharge pipe at the end of the 20 minute flushing period in relation to the laboratory derived concentration levels of effect are presented in Tables 11 and 12. In both tables the end-of-pipe concentrations are based on a range of well pumping rates and an azamethiphos target bath concentration of 100 mg/L ( $\equiv$ ppm). The effects concentrations are LC<sub>50</sub> and NOEC lethal values for Salmosan<sup>®</sup>, with active ingredient azamethiphos.

Table 11: The ratio  $(\overline{C}_{eop}/C_{loe})$  of the estimated average concentration  $(\overline{C}_{eop})$  of Salmosan® during flushing, expressed as azamethiphos, at the end of the flushing pipe to the level of effect values  $(C_{loe})$  for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of azamethiphos and organism exposures of 1 h and 10 d. The well volume is assumed to be 330  $m^3$  and the treatment concentration azamethiphos is assumed to be 100  $\mu$ g/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect		Ratio $(\bar{C}_{eop}/C_{loe})$				
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		С	<sub>loe</sub> = 1-h L0	C <sub>50</sub> lethal	(µg/L)		
Lobster I	>86.5	0.310	0.363	0.435	0.533	0.670	0.867
Lobster Adults	24.8	1.080	1.266	1.516	1.858	2.337	3.024
Mysids	>85.5	0.313	0.367	0.440	0.539	0.678	0.877
Crangon	>85.5	0.313	0.367	0.440	0.539	0.678	0.877
		$C_{lo}$	<sub>oe</sub> = 10-d L	.C <sub>50</sub> letha	l (μg/L)		
Lobster Adults	0.216	124	145.4	174.1	213.3	268.3	347.2
		C <sub>Ic</sub>	<sub>e</sub> = 1-h NC	DEC letha	al (µg/L)		
Lobster I	<0.37	72.4	84.9	101.6	124.5	156.6	202.7
Lobster Adults	9.85	2.7	3.2	3.8	4.7	5.9	7.6
Mysids	<0.97	27.6	32.4	38.8	47.5	59.7	77.3
Crangon	<0.97	27.6	32.4	38.8	47.5	59.7	77.3
		C <sub>loe</sub>	<sub>s</sub> = 10 d NO	OEC leth	al (µg/L)		
Lobster Adults	<0.123	217.7	255.3	305.7	374.6	471.1	609.7

Table 12: The ratio ( $C_{eop}$  (t =  $t_{fl}$ ) /  $C_{loe}$ ) of the estimated concentration of Salmosan<sup>®</sup>, expressed as azamethiphos, at the end of the flushing discharge pipe at the end of the flushing period ( $C_{eop}$  (t =  $t_{fl}$ )) to the  $C_{loe}$  values for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of azamethiphos and organism exposures of 1 h and 10 d. The well volume is assumed to be 330 m³ and the treatment concentration azamethiphos is assumed to be 100 µg/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect	Ratio $(C_{eop}(t = t_{fl}) / C_{loe})$					
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		С	i <sub>loe</sub> = 1-h L0	C <sub>50</sub> lethal	(µg/L)		
Lobster I	>86.5	0.030	0.056	0.102	0.188	0.344	0.631
Lobster Adults	24.8	0.106	0.195	0.357	0.655	1.200	2.200
Mysids	>85.5	0.031	0.056	0.104	0.190	0.348	0.638
Crangon	>85.5	0.031	0.056	0.104	0.190	0.348	0.638
		$C_{lo}$	<sub>oe</sub> = 10-d L	C <sub>50</sub> letha	l (µg/L)		
Lobster Adults	0.216	12.1	22.4	41.0	75.1	137.8	252.5
		$C_{lo}$	<sub>e</sub> = 1-h NC	DEC letha	al (µg/L)		
Lobster I	<0.37	7.1	13.1	23.9	43.9	80.4	147.4
Lobster Adults	9.85	0.3	0.5	0.9	1.6	3.0	5.5
Mysids	<0.97	2.7	5.0	9.1	16.7	30.7	56.2
Crangon	<0.97	2.7	5.0	9.1	16.7	30.7	56.2
	C <sub>loe</sub> = 10-d NOEC lethal (μg/L)						
Lobster Adults	<0.123	0.009	0.016	0.030	0.054	0.099	0.182

All of the time averaged ratios ( $\bar{C}_{eop}/LC_{50}$ ) for adult lobsters are near or slightly greater than one, indicating that the end-of-pipe concentrations are a little greater than the 1-h LC<sub>50</sub> values (Table 11). All of the ratios for the other test organisms are less than one indicating that the azamethiphos being flushed from the wells is just below the toxic 1-h LC<sub>50</sub> threshold. All of the  $\bar{C}_{eop}/NOEC$  ratio values are greater than one, indicating that the end-of-pipe concentrations are greater than the NOEC values reported by Burridge (2013). The ratios for adult lobsters are all

between 2.7 and 7.6, indicating the toxicity of the concentration of azamethiphos remaining in the well after flushing is not as great for adult lobsters as for the other test organisms.

With the exception of the 600 and 1200 m³/h pumping rates for adult lobsters, all of the  $C_{eop}$  ( $t = t_{fl}$ ) /  $LC_{50}$  ratio values are less than one (Table 12). This indicates that the end-of-pipe concentrations at the end of flushing are generally less than the  $LC_{50}$  values reported by Burridge (2013). However, most of the  $C_{eop}$  ( $t = t_{fl}$ ) / 1-h NOEC ratios are greater than one, only those for adult lobsters and pumping rates of 3600-2400 m³/s are not. These latter ratios are slightly less than one. This suggests that the discharged water will have some degree of effect on the non-target organisms.

The flushing times needed to reduce the end-of-pipe azamethiphos concentrations to the Burridge (2013)  $LC_{50}$  lethal values ( $t_{LC50}$ ) are presented in Table 13. With the exception of the adult lobsters at a pumping rate of 600 m<sup>3</sup>/s all of the 1-h  $t_{LC50}$  values were less than the typical treatment flushing periods, indicating that treatment flushing is generally sufficient to reduce the concentrations below the effects levels. The flushing times for 10-d  $LC_{50}$ s were considerably greater than zero and greater than typical 20 minute operational flushing times. This suggests that the operational flushing times need to extend beyond the 20 minute operational flushing time if the concentration is to be reduced below the 1-h  $LC_{50}$  threshold. The times for most of the 1-h NOEC levels were longer than the operational flushing times.

Table 13: Duration of flushing needed for end-of-flushing-pipe concentration for Salmosan<sup>®</sup>, expressed as azamethiphos, to be reduced to  $LC_{50}$  or NOEC values for various organisms and pumping rates. The time to reach the  $LC_{50}$  was calculated using  $t_{LC50}$  = -Ln ( $LC_{50}$  /  $C_{0}$ ) V / Q. The level of effect values are from Burridge (2013) and are based on measured concentrations of azamethiphos and organism exposures of 1 h and 10 d. The well volume (V) is assumed to be 330 m³ and the treatment concentration of azamethiphos is assumed to be 100 µg/L. Shaded cells in the table indicate the flushing times needed to dilute to the treatment concentration to the  $LC_{50}$  are greater than the operational flushing times ( $t_{LC50}$ ) >  $t_{flush}$ ). Typical operational flushing times are 20 minutes.

Organism	Level of Effect	Time (min) to Levels of Effect					
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		C	C <sub>loe</sub> = 1-h L	C <sub>50</sub> letha	l (µg/L)		
Lobster I	>86.5	1	1	1	2	2	5
Lobster Adults	24.8	8	9	12	15	23	46
Mysids	>85.5	1	1	1	2	3	5
Crangon	>85.5	1	1	1	2	3	5
		С	<sub>loe</sub> = 10-d I	_C <sub>50</sub> letha	al (µg/L)		
Lobster Adults	0.216	34	41	51	68	101	203
		C	<sub>oe</sub> = 1-h N(	DEC leth	al (µg/L)		
Lobster I	<0.37	31	37	46	62	92	185
Lobster Adults	9.85	13	15	19	25	38	76
Mysids	<0.97	25	31	38	51	76	153
Crangon	<0.97	25	31	38	51	76	153
	C <sub>loe</sub> = 10-d NOEC lethal (μg/L)						
Lobster Adults	<0.123	37	44	55	74	111	221

# End-of-Pipe: Alphamax®

The ratios of the concentration of deltamethrin at the end of the discharge pipe averaged over the flushing duration and the concentration at the end of the discharge pipe at the end of the 20 minute flushing period in relation to the laboratory derived concentration levels of effect are presented in Tables 14 - 17. In both tables the end-of-pipe concentrations are based on a range of well pumping rates and a deltamethrin target bath concentration of 2000 ng/L. The

effects concentrations are LC<sub>50</sub> and NOEC lethal values for Alphamax<sup>®</sup>, with active ingredient deltamethrin. The effects thresholds are from Burridge (2013).

Table 14: The ratio( $\overline{C}_{eop}/C_{loe}$ ) of the estimated average concentration ( $\overline{C}_{eop}$ ) of Alphamax<sup>®</sup> during flushing, expressed as deltamethrin, at the end of the flushing pipe to the LC<sub>50</sub> level of effect values ( $C_{loe}$ ) for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, 24 h, and 10 d. The well volume is assumed to be 330 m³ and the treatment concentration deltamethrin is assumed to be 2000 ng/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect			Ratio ( $\bar{C}_{e}$	op/C <sub>loe</sub> )		
Pumping rate (Q) m <sup>3</sup> /h and (% of 3000 m <sup>3</sup> /h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		C <sub>loe</sub> =	= 1-h LC <sub>50</sub> l	ethal (ng/l	_)		
Lobster I	3.4	157.5	184.7	221.2	271.0	340.9	441.1
Lobster III*	36.5	14.7	17.2	20.6	25.2	31.8	41.1
Lobster Adults	18.8	28.5	33.4	40.0	49.0	61.6	79.8
Mysids	13.9	38.5	45.2	54.1	66.3	83.4	107.9
Crangon**	142.0	3.8	4.4	5.3	6.5	8.2	10.6
		C <sub>loe</sub> =	24-h LC <sub>50</sub>	lethal (ng/	L)		
Lobster I	0.8	669	785	940	1152	1449	1875
Lobster II	0.6	893	1047	1253	1536	1932	2500
Lobster IV	1.7	315	369	442	542	682	882
Lobster Adults	15.0	36	42	50	61	77	100
Mysids	1.4	383	449	537	658	828	1071
Crangon	27.0	20	23	28	34	43	56
	$C_{loe}$ = 10-d LC <sub>50</sub> lethal (ng/L)						
Lobster Adults	14.7	36.4	42.7	51.2	62.7	78.8	102.0

<sup>\*</sup> based on nominal effects concentrations 1 h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

<sup>\*\*</sup> nominal effects concentrations from Fairchild et al. (2010)

Table 15: The ratio ( $\overline{C}_{eop}/C_{loe}$ ) of the estimated average concentration ( $\overline{C}_{eop}$ ) of Alphamax<sup>®</sup> during flushing, expressed as deltamethrin, at the end of the flushing pipe to the NOEC level of effect values ( $C_{loe}$ ) for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, 24 h, and 10 d. The well volume is assumed to be 330 m³ and the treatment concentration deltamethrin is assumed to be 2000 ng/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect			Ratio (C	eop/C <sub>loe</sub> )				
Pumping rate (Q) m <sup>3</sup> /h and (% of 3000 m <sup>3</sup> /h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)		
		C <sub>loe</sub> = 1-h NOEC lethal (ng/L)							
Lobster I	<0.6	893	1047	1253	1536	1932	2500		
Lobster Adults	3.6	149	174	209	256	322	417		
Mysids	0.9	595	698	836	1024	1288	1667		
		C <sub>loe</sub> =	= 24-h NOE	C lethal (ı	ng/L)				
Lobster I	<0.08	6694	7852	9399	11518	14488	18748		
Lobster II	<0.08	6694	7852	9399	11518	14488	18748		
Lobster IV	<0.08	6694	7852	9399	11518	14488	18748		
Lobster Adults	<4.8	112	131	157	192	241	312		
Mysids	<0.2	2678	3141	3760	4607	5795	7499		
Crangon	<5	107	126	150	184	232	300		
		$C_{loe} = 2$	24-h NOEC	sublethal	(ng/L)				
Lobster I	<0.08	6694	7852	9399	11518	14488	18748		
Lobster II	<0.08	6694	7852	9399	11518	14488	18748		
Lobster IV	<0.08	6694	7852	9399	11518	14488	18748		
Lobster Adults	<0.06	8925	10469	12533	15357	19317	24998		
Mysids	<0.2	2678	3141	3760	4607	5795	7499		
Crangon	<8	67	79	94	115	145	187		
		C <sub>loe</sub> = 10-d NOEC lethal (ng/L)							
Lobster Adults	5	107	126	150	184	232	300		

Table 16: The ratio ( $C_{eop}$  ( $t=t_{fl}$ ) /  $C_{loe}$ ) of the estimated concentration of Alphamax<sup>®</sup>, expressed as deltamethrin, at the end of the flushing discharge pipe at the end of the flushing period ( $C_{eop}$  ( $t=t_{fl}$ )) to the LC<sub>50</sub>  $C_{loe}$  values for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin. The well volume is assumed to be 330 m³ and the treatment concentration deltamethrin is assumed to be 2000 ng/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect	Ratio (C <sub>eop</sub> (t=t <sub>fl</sub> ) / C <sub>loe</sub> )					
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		$C_{lo}$	<sub>pe</sub> = 1-h LC <sub>5</sub>	<sub>50</sub> lethal (n	g/L)		
Lobster I	3.4	15.499	28.412	52.085	95.483	175.038	320.880
Lobster III*	36.5	1.44	2.65	4.85	8.89	16.30	29.89
Lobster Adults	18.8	2.80	5.14	9.42	17.27	31.66	58.03
Mysids	13.9	3.79	6.95	12.74	23.36	42.82	78.49
Crangon**	142.0	0.37	0.68	1.25	2.29	4.19	7.68
		$C_{lo}$	<sub>e</sub> = 24-h LC	5 <sub>50</sub> lethal (i	ng/L)		
Lobster I	0.8	65.9	120.8	221.4	405.8	743.9	1363.7
Lobster II	0.6	87.8	161.0	295.2	541.1	991.9	1818.3
Lobster IV	1.7	31.0	56.8	104.2	191.0	350.1	641.8
Lobster Adults	15.0	3.5	6.4	11.8	21.6	39.7	72.7
Mysids	1.4	37.6	69.0	126.5	231.9	425.1	779.3
Crangon	27.0	2.0	3.6	6.6	12.0	22.0	40.4
	$C_{loe}$ = 10-d LC <sub>50</sub> lethal (ng/L)						
Lobster Adults	14.7	3.58	6.57	12.05	22.08	40.49	74.22

<sup>\*</sup> based on nominal effects concentrations 1 h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

<sup>\*\*</sup> nominal effects concentrations from Fairchild et al. (2010)

Table 17: The ratio ( $C_{eop}$  ( $t = t_{fl}$ ) /  $C_{loe}$ ) of the estimated average concentration ( $C_{eop}$ ) of Alphamax<sup>®</sup>, at the end of the flushing discharge pipe at the end of the flushing period ( $C_{eop}$  ( $t = t_{fl}$ )) to the NOEC level of effect values ( $C_{loe}$ ) for various organisms and pumping rates. The  $C_{loe}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, 24 h, and 10 d. The well volume is assumed to be 330 m³ and the treatment concentration deltamethrin is assumed to be 2000 ng/L. A typical operational flushing time of 20 minutes was assumed. Shaded cells in the table highlight ratio values greater than one and indicate a potential for toxic effects.

Organism	Level of Effect		R	Ratio (C <sub>eop</sub>	$(t = t_{fl}) / C_{lo}$	e)	
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
		C	G <sub>loe</sub> = 1-h NO	EC lethal (i	ng/L)		
Lobster I	0.6	87.8	161.0	295.2	541.1	991.9	1818.3
Lobster Adults	3.6	14.6	26.8	49.2	90.2	165.3	303.1
Mysids	0.9	58.6	107.3	196.8	360.7	661.3	1212.2
		C	<sub>loe</sub> = 24-h NC	EC lethal (	(ng/L)		
Lobster I	<0.08	659	1208	2214	4058	7439	13637
Lobster II	<0.08	659	1208	2214	4058	7439	13637
Lobster IV	<0.08	659	1208	2214	4058	7439	13637
Lobster Adults	4.8	11	20	37	68	124	227
Mysids	<0.2	263	483	885	1623	2976	5455
Crangon	5	11	19	35	65	119	218
		C	<sub>loe</sub> = 24-h NC	EC lethal (	(ng/L)		
Lobster I	<0.08	659	1208	2214	4058	7439	13637
Lobster II	<0.08	659	1208	2214	4058	7439	13637
Lobster IV	<0.08	659	1208	2214	4058	7439	13637
Lobster Adults	<0.06	878	1610	2952	5411	9919	18183
Mysids	<0.2	263	483	885	1623	2976	5455
Crangon	<8	7	12	22	41	74	136
		C	oe = 10-d NC	EC lethal (	(ng/L)		
Lobster Adults	5	11	19	35	65	119	218

All of the time averaged ratios ( $\bar{C}_{eop}/LC_{50}$  and  $C_{eop}$  / NOEC), indicate that the end-of-pipe concentrations are much greater than the LC<sub>50</sub> and NOEC values (Tables 14 –17). This indicates that Alphamax<sup>®</sup> being flushed from the wells is toxic at the LC<sub>50</sub> and NOEC levels to the ambient waters and that the concentration of deltamethrin in the water remaining in the well after flushing is greater than the NOEC lethal effects concentrations.

The flushing times needed to reduce the end-of-pipe concentration to the Burridge (2013)  $LC_{50}$  and NOEC lethal values ( $t_{LC50}$ ) for the above Alphamax<sup>®</sup> treatments are presented in Tables 18 and 19. As expected, most times are greater than the operational flushing times of 20 minutes.

Table 18: Duration of flushing time needed for the end-of-flushing-pipe concentration for Alphamax<sup>®</sup>, expressed as deltamethrin, to be reduced to  $LC_{50}$  values for various organisms and pumping rates. The time to reach the  $LC_{50}$  was calculated using  $t_{LC50}$  = -Ln ( $LC_{50}$  /  $C_{0}$ ) V / Q. The  $LC_{50}$  values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, 24 h, and 10 d. The well volume (V) is assumed to be 330 m³ and the treatment concentration of deltamethrin is assumed to be 2000 ng/L. Shaded cells in the table indicate the flushing times needed to dilute to the treatment concentration to the  $LC_{50}$  are greater than the operational flushing times ( $t_{LC50}$ ) t<sub>flush</sub>). Typical operational flushing times are 20 minutes.

Organism	Level of Effect	Time (min) to LC <sub>50</sub>					
Pumping rate (Q) m³/h and (% of 3000 m³/h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
			1-h LC <sub>50</sub> le	thal (ng/L	)		
Lobster I	3.4	35	42	53	70	105	210
Lobster III*	36.5	22	26	33	44	66	132
Lobster Adults	18.8	26	31	39	51	77	154
Mysids	13.9	27	33	41	55	82	164
Crangon**	142.0	15	17	22	29	44	87
			24-h LC <sub>50</sub> le	ethal (ng/L	_)		
Lobster I	0.8	43	52	65	86	129	258
Lobster II	0.6	45	54	67	89	134	268
Lobster IV	1.7	39	47	58	78	117	233
Lobster Adults	15.0	27	32	40	54	81	161
Mysids	1.4	40	48	60	80	120	240
Crangon	27.0	24	28	36	47	71	142
	10-d LC <sub>50</sub> lethal (ng/L)						
Lobster Adults	14.7	27	32	41	54	81	162

<sup>\*</sup> based on nominal effects concentrations 1 h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

<sup>\*\*</sup> nominal effects concentrations from Fairchild et al. (2010)

Table 19: Duration of flushing needed for end-of-flushing-pipe concentration for Alphamax<sup>®</sup>, expressed as deltamethrin, to be reduced to NOEC values for various organisms and pumping rates. The time to reach the  $LC_{50}$  was calculated using NOEC = -Ln (NOEC /  $C_0$ ) V / Q. The NOEC values are from Burridge (2013) and are based on measured concentrations of deltamethrin and organism exposures of 1 h, 24 h, and 10 d. The well volume (V) is assumed to be 330 m³ and the treatment concentration of deltamethrin is assumed to be 2000 ng/L. Shaded cells in the table indicate the flushing times needed to dilute to the treatment concentration to the NOEC levels are greater than the operational flushing times (NOEC >  $t_{flush}$ ). Typical operational flushing times are 20 minutes.

Organism	Level of Effect			Time (min)	to LC <sub>50</sub>		
Pumping rate (Q) m <sup>3</sup> /h and (% of 3000 m <sup>3</sup> /h)		3600 (120%)	3000 (100%)	2400 (80%)	1800 (60%)	1200 (40%)	600 (20%)
			1-h NOEC I	ethal (ng/L)			
Lobster I	<0.6	>45	>54	>67	>89	>134	>268
Lobster Adults	3.6	35	42	52	70	104	209
Mysids	0.9	42	51	64	85	127	254
			24-h NOEC	lethal (ng/L)			
Lobster I	<0.08	>56	>67	>84	>111	>167	>334
Lobster II	<0.08	>56	>67	>84	>111	>167	>334
Lobster IV	<0.08	>56	>67	>84	>111	>167	>334
Lobster Adults	4.8	33	40	50	66	100	199
Mysids	<0.2	>51	>61	>76	>101	>152	>304
Crangon	5	33	40	49	66	99	198
		2	4-h NOEC sı	ublethal (ng/l	_)		
Lobster I	<0.08	56	67	84	111	167	334
Lobster II	<0.08	56	67	84	111	167	334
Lobster IV	<0.08	56	67	84	111	167	334
Lobster Adults	<0.6	57	69	86	115	172	344
Mysids	<0.2	51	61	76	101	152	304
Crangon	<8	30	36	46	61	91	182
	10-d NOEC lethal (ng/L)						
Lobster Adults	5	33	40	49	66	99	198

#### WELL-BOAT: TOXICITY OF FLUSHING DISCHARGE JET

The water containing the therapeutant exits the discharge pipe and forms a turbulent jet. As described in Page et al. (2014), the steady state concentration of the rapeutant within the jet has a maximum along the main axis of the jet and this maximum ( $C_{max}$ ) is approximated by the relationship

$$C_{max} = (5d / x) C_{ep}$$

where *d* is the diameter of the discharge pipe, *x* is the distance along the main discharge axis, i.e., the distance perpendicular to the well-boat and  $C_{ep}$  is the concentration of therapeutant at the end of the discharge pipe. In the case of well-boats the end-of-pipe concentration is time varying and is estimated as

$$C_{ep}(t) = C_0 \exp(-Qt / V)$$

where  $C_0$  is the target concentration within the well estimated as  $C_0 = M / V$ . Combining the above equations gives

$$C_{max} = (5d / x) C_0 \exp(-Qt / V)$$
  
or  
 $C_{max} = (5d / x) (M / V) \exp(-Qt / V).$ 

Although this is the combination of a steady state jet dynamic and a time varying discharge concentration, the observations shown in Page et al. (2014) indicate it gives a reasonable first

approximation to the discharge concentrations.

The dilution factor for the therapeutant as a function of distance from the discharge pipe and time since the beginning of the well flushing is given by rearranging the above relationship as follows

$$Df = \frac{C_{\text{max}}}{C_0} = \frac{C_{\text{max}}V}{M} = \frac{5d}{x} \exp(-Qt/V)$$

The relationship indicates that the concentration of therapeutant in the flushing discharge from well-boats can be expected to be diluted by a factor of 10-100 within a few tens to a few hundred metres of the well-boat.

The distance from the well-boat (i.e., x) at which the maximum therapeutant concentration equals a particular level of effect such as an LC<sub>50</sub> or level of no effect (NOEC) is given by setting  $C_{max}$  = effect\_level =  $(5d / x) (M / V) \exp (-Qt / V)$  and rearranging so

$$x(t) = \frac{5d (M/V) \exp(-Qt/V)}{effect level}$$

This equation indicates that the distance at which the concentration equals the level of effect reduces with time because the end-of-pipe concentration is reducing with time. This relationship, however, is not representative of the first few seconds or minutes of the discharge because the discharge needs to form before the above relationships apply. An equation describing this initial temporal evolution of the jet has not been determined yet.

The time at which the maximum therapeutant concentration at a particular distance from the well-boat equals a specified level of effect, such as an LC<sub>50</sub> or level of no effect (NOEC), is given by setting  $C_{max} = effect\_level = (5d / x) (M / V) \exp (-Qt / V)$  and rearranging so

$$t_{effect} = -\frac{V}{Q} Ln \left( \frac{C_{loe} xV}{5dM} \right)$$
 or 
$$t_{effect} = -\frac{V}{Q} Ln \left( \frac{C_{loe} x}{5dC_0} \right)$$

# Flushing Discharge Jet: Paramove<sup>®</sup>50

Since the concentrations of hydrogen peroxide at the end-of-the-pipe during and at the end of the discharge period were essentially non-toxic or only weakly toxic, the concentrations of Paramove<sup>®</sup>50 in the discharge jet would be diluted by another factor of 10-100 which would make them non-toxic by these indicators.

# Flushing Discharge Jet: Salmosan®

The concentrations of azamethiphos at the end of the pipe during and at the end of the discharge period were essentially non-toxic or only weakly toxic, in terms of  $LC_{50}$ s and the concentrations of azamethiphos in the discharge jet would be diluted by another factor of 10-100 which would make them for the most part non-toxic by these indicators. For the most part the same holds true when the NOEC thresholds are considered.

## Flushing Discharge Jet: Alphamax®

The concentrations of Alphamax<sup>®</sup> are less likely to be diluted by the discharge jet to below LC<sub>50</sub> and NOEC thresholds. Efforts to calculate these dilutions and the distances from the well-boat at which the concentrations are below the toxicity thresholds have not yet been determined.

#### SUMMARY

The threshold results presented by Burridge (2013) considered in concert with predictions of dispersions specific to southwest New Brunswick (Page et al. 2014) have resulted in predictions of potential degrees of toxicity as well as length scales and areas of impact. The data clearly show that potential effects are product specific. AlphaMax® is more lethal than Salmosan® which is more lethal than Paramove®50 to the species tested in these studies. These relative toxicities are also reflected by the recommended treatment concentrations, i.e., high concentrations of Paramove®50 are needed to kill sea lice, whereas lower concentrations are needed for Salmosan®, and even lower for Alphamax®.

The approach taken in this report is one way of combining estimates of exposures and toxicity thresholds and it gives some indication of the *in situ* potential for toxic effects. As indicated in the introduction, this is a work in progress and other approaches have not been identified, but may be considered if warranted as more appropriate or accurate.

To summarize the potential exposure results, the focus was placed on the ratios of therapeutant exposure concentration to the one hour exposure level of effects concentration and on the most toxic results for each organism and therapeutant examined from the tables presented.

Table 20 summarizes the ratios for each of the therapeutants at treatment concentration, which represents the initial discharge exposure. The 1-h effects exposures were used since the durations are most similar to those likely to be experienced *in situ* by sessile organisms.

Table 20: Summary of relative toxicity of Paramove® 50, Salmosan® and Alphamax® at treatment concentration to effects concentration for various organisms. The C<sub>loe</sub> values are from Burridge (2013) and are based on measured concentrations of hydrogen peroxide, azamethophos, and deltamethrin, respectively.

Organism		Ratio ( $\overline{C}_0/C_{loe}$ )	Ratio ( $\bar{C}_0/C_{loe}$ )						
	Paramove® 50	Salmosan®	Alphamax®						
	C	$C_{loe}$ = 1-h LC <sub>50</sub> lethal (mg/L)							
Lobster Stage I	0.7	<0.9	588.2						
Lobster Adults	<0.3	2.5	106.4						
Mysids	1.2	<0.9	143.9						
Crangon	0.4	<0.9	14.1						
	Clos	= 1-h NOEC lethal (mg	/L)						
Lobster Stage I	3.4	>270.0	>3333.3						
Lobster Adults	1.2	10.2	555.5						
Mysids	>4.9	>103.0	2222.2						
Crangon	>5.4	>103.0	NA*						

<sup>\*</sup>denotes not available

The ratios were then grouped into three main categories. Ratios less than 0.1 indicate the exposure has relatively little effect on the non-target organism. Ratios greater than 0.1 and less than 10 are considered to indicate the exposures are border line between having relatively little effect to having some effect. Ratios greater than 10 are considered to have a strong potential for effects. The summaries are shown in Table 21. In these tables, ratios less than 0.1 are green, ratios between 0.1 and 10 are grey and ratios greater than 10 are red. In tables 22 and 23, the magnitude of the ratio in the red cells is indicated by the number of dots (•). Two dots indicate ratios are in the hundreds, three dots in the thousands and so on. We have also grouped the larval results into one category. Within each category of non-target organism (lobster larvae, lobster adult, mysid and *Crangon*) and effects indicator (1-h LC<sub>50</sub> lethal or 1-h NOEC lethal) the ratio assignment corresponds to the largest ratio estimated for the category. This approach emphasises the most toxic results and accommodates, to some extent, the inherent variation in toxicity results and estimated exposures.

Table 21: Summary of the estimated effect of therapeutant tarp treatment concentration on non-target organisms as indicated by the ratio of exposure concentration to the 1-h  $LC_{50}$  or 1h NOEC thresholds of effect concentration. The colour of the cells indicates the level of effect. Grey cells indicate the ratios are between 0.1 and 10. Red cells indicate the ratios were greater than 10. Red cells with \*\* indicate the ratios are 10-100, red cells with \*\*\* indicate the ratios are greater than 100-1000 and red cells with \*\*\*\* indicate the ratios are greater than 1000. The numbers under each therapeutant are the assumed treatment concentrations.

		Therapeutant  Treatment Concentration  Level of Effect						
	Paramo 1200		Salmo			namax <sup>®</sup> 00 ng/L		
Non-Target Organism	NOEC	LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	LC <sub>50</sub>		
End-of-Pipe or edge	of cage fo	or tarp trea	atments					
Lobster larvae			••		••••	••••		
Adult lobster					••••	••		
Mysids			••		••••	••		
Crangon					•••	••		
End-of-Pipe for well-	boat treat	ments						
Lobster larvae			••		••••	••••		
Adult lobster					•••	••		
Mysids			••		••••	••		
Crangon			••		na			

The summary of the effects estimates for the treatment concentrations themselves (Table 21) indicate that Paramove®50 treatment concentrations are marginally toxic, Salmosan® treatment concentrations are somewhat toxic and AlphaMax® concentrations are highly toxic. This is not surprising since the treatment concentrations are designed for the most part to kill or disable the target crustacean, sea lice.

A similar consideration for the edge-of-cage and end-of-pipe is shown in Table 21. Although the table does not show a reduction in the effect for Paramove®50, this is because the grey effect category is broad and does not reflect the 10x or so reduction in the toxicities produced by the flushing.

Table 22: Summary of elapsed time and end of plume distance traveled to reach effect level for various organisms following tarp treatment with three different therapeutants. Effect levels are from Burridge (2013), and Fairchild et al. (2010), as noted previously in the text. In the table CE stands for cage edge.

	Therapeutant  Treatment Concentration  Elapsed Time and Distance from Tarp							
	Salı	Treatment Release to Reach Effect Level  Salmosan® Alphamax® 2000 ng/L						
Non-Target Organism	Time (hours)	Distance (m)	Time (hours)	Distance (m)				
1-h LC <sub>50</sub>								
Lobster larvae	0	CE	6.7	3010				
Adult lobster	0.47	170	3.36	1463				
Mysids	0	CE	3.82	1671				
Crangon*	0	CE	1.23	534				
	1-h NOEC	;						
Lobster larvae	4.95	1780	12.72	6001				
Adult lobster	1.0	1.0 359 6.55						
Mysids	3.3	3.3 1193 10.57 4908						
Crangon	3.3	1193	n/a	n/a				

<sup>\*</sup> Data from Fairchild et al. (2010).

The zone of potential effect from Salmosan® net-pen treatments based on 1-h  $LC_{50}$  values is in the order of 10s of metres to about 200 m whereas the zone for Alphamax® treatments is 100's of metres to several kilometres (Table 22). The zones when they are based on the 1-h lethal NOEC values are of order 100s of meters to a couple of kilometers for Salmosan® and several kilometres for Alphamax®.

Using the location of salmon aquaculture sites in southwest New Brunswick, an estimate of potential overlapping zones of influence following bath treatments can be made (Figure 1). This shows the potential for therapeutants in the discharge plume, assuming a circular zone of influence of 2 kilometres, to overlap should multiple treatments occur in a given area, as well as where there may be potential for discharge plumes to interact with the shallow near-shore or intertidal areas. However, more work will be required to refine these estimates, including the influence of site specific influences such as effect of tides and the distribution of sensitive species on exposure and consequence estimates.

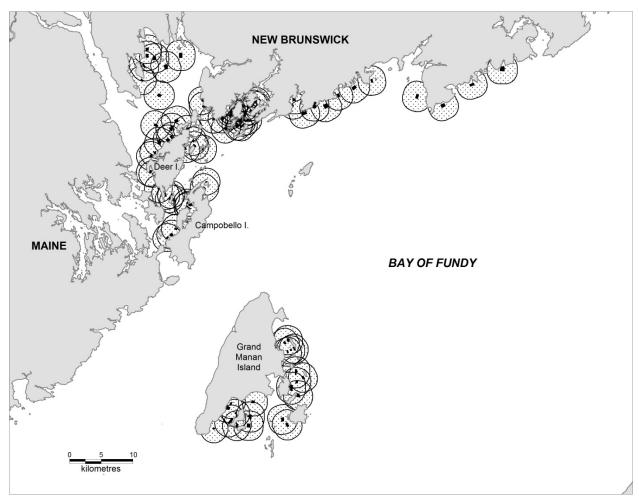


Figure 1: Two kilometre circular zones of influence surrounding aquaculture locations in southwest New Brunswick as an estimate of individual and cumulative potential zones of impact.

#### **OVERALL CONCLUSIONS**

The end-of-pipe concentrations resulting from Paramove<sup>®</sup>50 bath treatments with target hydrogen peroxide (the active ingredient) concentrations of 1200 mg/L are likely to result in only limited hazardous effects to stage I lobster larvae, adult lobsters, Mysids and Crangon based on the 1-h LC<sub>50</sub> lethal results. Furthermore, the concentrations of hydrogen peroxide in flushing discharge jets and subsequent plumes are not toxic by these criteria. The discharge of the residual hydrogen peroxide from the wells during the pumping of fish back into cages is also not hazardous to these organisms. Threshold concentrations are reached at "end-of-pipe".

The end-of-pipe concentrations resulting from Salmosan bath treatments with target azamethiphos concentrations of 100  $\mu$ g/L are likely to result in some potential hazardous effects to stage I lobster larvae, adult lobsters, Mysids and Crangon based on the 1-h LC<sub>50</sub> results. The concentrations in the discharge jets are likely to cause only limited potential effects. The dispersion plume from a single treatment may contain lethal concentrations of azamethiphos for minutes to about an hour and at distances measured in a hundreds of metres.

The end-of-pipe concentrations resulting from AlphaMax $^{\otimes}$  bath treatments with target deltamethrin concentrations of 2000 ng/L are likely to result in lethal effects to stage I, II, III and IV lobster larvae, adult lobsters, Mysids and Crangon based on 1-h LC<sub>50</sub> results. The dispersion plume may contain lethal concentrations of deltamethrin for several hours after release and at distances in the order of 10 km.

As noted above there are differences in sensitivity amongst the tested species and life stages (Burridge 2013).

Whether the potential for impact to non-targets is realized in the field depends on whether the sensitive species and/or life stage (e.g., stage I American lobsters), is present within the zone of influence at the time of the treatment. A summary of the time of year when various organisms are present in southwest New Brunswick waters can be found in Burridge (2013, see Table 13). Benthic species such as *Crangon* and juvenile and adult American lobster will only be exposed when the effluent plume reaches the bottom. While these considerations are important, they have not been included in this analysis. As the process of refining these models and predictions moves forward, details including life-cycles and the ecology of non-target organisms will need to be incorporated.

The calculations presented are based on initial and measured concentrations, in this case the prescribed treatment concentration. Should the starting concentration change for whatever reason, binding to organics or net fouling, chemical breakdown, etc., the prediction of consequences to non-target organisms will also change, presumably resulting in lower risk. However, these processes will also affect the exposure of the target organism, sea lice, and possibly result in lower efficacy.

#### **ACKNOWLEDGEMENTS**

Blythe Chang prepared Figure 1. Monica Lyons and Ingrid Burgetz helped with the manuscript revisions.

#### REFERENCES

- Burridge, L.E., Haya, K., Page, F.H., Waddy, S.L., Zitko, V., and Wade, J. 2000. The lethality of the cypermethrin formulation Excis® to larval and post-larval stages of the American lobster (*Homarus americanus*). Aquaculture 182(2000): 37-47.
- Burridge, L. 2013. 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. DFO Can. Sci. Advis. Sec. Res. Doc. 2013/050. iv + 25 p.
- Crane, M., Gross, M., Maycock, D.S., Grant, A. and Fossum, B.H. 2011. Environmental quality standards for a deltamethrin sea louse treatment in marine finfish aquaculture based on survival time analyses and species sensitivity distributions. Aquaculture Research, 42(2): 68-72.
- Ernst, W, Jackman, P., Doe, K., Page, F., Julien, G., MacKay, K., and Sutherland, T. 2001. Dispersion and toxicity to non-target aquatic organisms of pesticides used to treat sea lice on salmon in net pen enclosures. Marine Pollution Bulletin 42(6): 433-444.
- Fairchild, W.L., Doe, K.D., Jackman, P.M., Arsenault, J.T., Aube, J.G., Losier, M., and Cook, A.M. 2010. Acute and chronic toxicity of two formulations of the pyrethroid pesticide deltamethrin to an amphipod, sand shrimp and lobster larvae. Can. Tech. Rep. Fish. Aquat. Sci. 2876: vi + 34 p.
- Okubo, A. 1974. Some speculations on oceanic diffusion diagrams. Rapp. P.-v. Réun. Cons. int. Explor. Mer 167: 77-85.
- Page, F.H., Chang, B.D., Beattie, M., Losier, R., McCurdy, P., Bakker, J., Haughn, K., Thorpe, B., Fife, J., Scouten, S., Bartlett G., and Ernst, B. 2014. 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. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/102.