DERIVATION OF MARINE EQS FOR EMAMECTIN BENZOATE

REPORT TO SCOTTISH SALMON PRODUCERS ORGANISATION AND MSD ANIMAL HEALTH, FROM WCA AND AG-HERA

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EXECUTIVE SUMMARY

Emamectin Benzoate (EMB) has been authorised in Scotland for use as a veterinary medicine to control sea lice in farmed salmon for almost 20 years.

Studies conducted by the Scottish Association for Marine Science (SAMS) have examined potential relationships between the use of sea lice medicines in Scottish lochs and effects on benthic communities of organisms. On this basis, the Scottish Environment Protection Agency (SEPA) commissioned a review of the Environmental Quality Standards (EQS) for EMB (WRc 2017).

No chronic ecotoxicity effect data were available for marine sediment organisms at the time of the EQS review, and a 'far-field' long-term marine sediment EQS for EMB was proposed based on a single long-term sediment study undertaken with the freshwater insect *Chironomus riparius.* The EQS value was derived using the No Observed Effect Concentration (NOEC) for the emergence of adult midges from pupae (1.175 µg kg^{-1} dry weight), to which an Assessment Factor (AF) of 100 was applied to give a PNEC of 0.012 µg kg^{-1}, or 12 ng kg^{-1} (dry weight) (WRc 2017).

Since the SEPA proposal for revised EQS, the Scottish Salmon Producers' Organisation (SSPO) and the manufacturer of the product containing EMB used for the control of fish lice (MSD Animal Health (MSD)) have commissioned a programme of new ecotoxicity testing for EMB in order to fill gaps in the marine ecotoxicity dataset. This testing covers both the acute and chronic effects of EMB on pelagic and benthic marine species.

This report updates the marine EQS for EMB to include the new data generated by SSPO and MSD. Short-term (Maximum Acceptable Concentration (MAC)-EQS) and long-term (Annual Average (AA)-EQS) have been developed for marine water, and 'far field' and 'near field' EQS for marine sediment.

All of the new studies were conducted according to the principles of GLP, within reputable Contract Research Organisations, and to internationally standardised guidelines (or accepted peer-reviewed methodologies where standardised guidelines are not available). All studies are reliable and relevant for EQS derivation.

In particular, significant gaps in the long-term sediment ecotoxicity dataset for EMB have been addressed, such that the reliable and relevant dataset now includes four studies undertaken with benthic marine organisms, encompassing three species.

We propose the following EQS, based on the expanded marine ecotoxicity dataset for EMB.

EQS	Value and units	Driving datapoint	Assessment Factor
Maximum Acceptable Concentration (MAC) for marine water	1.1 ng L^{-1}	Geometric mean LC50 (n=3) for *Americamysis bahia* of 56 ng L^{-1}	50
Annual Average (AA) EQS for marine water	0.47 ng L^{-1}	EC10 for *Americamysis bahia* growth of 9.44 ng L^{-1}	20
'Far field' sediment EQS	1290 ng kg^{-1} (dry weight) 997 ng kg^{-1} (wet weight)	EC10 for *Arenicola marina* casting of 12900 ng kg^{-1} (dry weight) 9969.1 ng kg^{-1} (wet weight)	10
'Near-field' sediment EQS	2580 ng kg^{-1} (dry weight) 1994 ng kg^{-1} (wet weight)	EC10 for *Arenicola marina* casting of 12900 ng kg^{-1} (dry weight) 9969.1 ng kg^{-1} (wet weight)	5

The proposed 'far field' sediment EQS is greater than that currently implemented by SEPA (763 ng kg^{-1} wet weight). The higher 'far field' sediment EQS derived here results from a dataset comprising studies that are highly relevant for the assessment of the potential hazards of EMB to sensitive groups of marine organisms, and an associated reduction in the AF applied. The currently implemented 'far field' EQS was based on a limited dataset of acute toxicity data, with a necessarily higher AF.

EMB acts as a chloride channel activator in arthropods, thus exerting a selective toxicity against sea lice. Therefore, in the marine environment, this mode of action means that crustaceans are likely to be amongst the most sensitive marine taxonomic groups to EMB. The chronic marine sediment dataset now contains reliable and relevant data for two benthic crustacean species (across three studies), and these highlight that the sensitivity of benthic crustaceans to sediment-bound EMB is much lower than the revised EQS proposed by SEPA (12 ng kg^{-1} dry weight). The most sensitive chronic endpoint for crustaceans (growth) is 17600 ng kg^{-1} dry weight, corresponding to a single-study EQS of 176 ng kg^{-1} dry weight (when applying the same AF of 100 as for the *C. riparius* study).

As there are sufficient reliable chronic ecotoxicity data for marine benthic organisms for EQS derivation, and this dataset includes two representatives of the taxonomic group (crustaceans) likely to be amongst the most sensitive to EMB based on its mode of action, we recommend that the marine sediment EQS are derived solely using marine species, and that AFs of 10 and 5 are applied to the lowest endpoint to derive the 'far field' and 'near field' EQS, respectively.

The EQS recommended here are proposed as environmentally responsible values, supported by reliable and sensitive laboratory ecotoxicity data. Further support for this endpoint is provided by the outcome of an extensive field data set.

CONTENTS

1 INTRODUCTION

EMB has been authorised in Scotland for use as a veterinary medicine to control sea lice in farmed salmon for almost 20 years. SEPA require a series of additional regulatory consent conditions on the use of EMB in Scottish aquaculture. One of these conditions is a requirement to comply with EQS for EMB in water and sediment near salmon pens. The EQS currently applied were derived by SEPA in the late 1990s and are considered achievable by the Scottish salmon industry, based on current practices and usage of EMB for the treatment of sea lice in salmon.

Studies conducted by The Scottish Association for Marine Science (SAMS) have examined potential relationships between the use of sea lice medicines in Scottish lochs and effects on benthic communities of organisms. The most recent of these studies (known as PAMP-2 (SARF 2016)) suggests a potential link between measured sediment concentrations of EMB and a decline in marine benthic crustacean communities. On this basis, SEPA commissioned a review of the EQS for EMB, and proposed new EQS for seawater and sediment. This review was conducted according to the Water Framework Directive (WFD) EQS Technical Guidance Document (TGD) (EC 2011).

In 2017, when the WRc EQS review was concluded, there were no chronic ecotoxicity effect data (e.g. growth or reproduction) available for marine sediment organisms. The EQS review therefore proposed a 'far field' long-term marine sediment EQS for EMB based on a single long-term sediment study undertaken with the freshwater insect *Chironomus riparius.* The larvae of *C. riparius* live and feed in freshwater sediments, but adults are not aquatic. In addition, the most sensitive endpoint in the *C. riparius* study was adult emergence from pupae (i.e. following metamorphosis from larvae). Since there are no truly marine insect species, nor any other marine invertebrate species which have life cycles involving aquatic larvae and non-aquatic adults, this study could be considered as not relevant for the derivation of a long-term marine sediment EQS for EMB.

The NOEC (emergence) used in the sediment EQS derivation was 1.175 µg EMB kg^{-1} dry weight, to which an AF of 100 was applied to give a 'far field' EQS of 0.012 µg kg^{-1}, or 12 ng kg^{-1} (dry weight) (WRc 2017).

In addition, a 'near-field' marine sediment EQS was developed, which used the same sediment NOEC (1.175 µg EMB kg^{-1} dry weight), but applied an AF of 10, giving a 'near-field' EQS of 120 ng kg^{-1} (dry weight) (WRc 2017).

Since the SEPA proposal for revised EQS was published, SSPO and MSD have commissioned a programme of new ecotoxicity testing for EMB in order to fill gaps in the marine ecotoxicity dataset. This testing covers both the acute and chronic effects of EMB on pelagic and benthic marine species.

This report updates the marine EQS for EMB to include the data generated by SSPO and MSD during 2018. Short-term (Maximum Acceptable Concentration (MAC)) and long-term (Annual Average (AA)) EQS have been developed for marine water, and 'far field' and 'near field' EQS have been derived for marine sediment.

In all cases, the 2017 EQS derivation (WRc 2017) has been used as the starting point for development, and the existing (pre-2018) data cited therein have been used without further assessment of their reliability, or review of the source reports and papers.

Section 2 of this report details the new EMB test data and provides an assessment of their reliability and relevance using the CRED approach (Moermond et al. 2016) recommended by the WFD EQS TGD (EC 2011). In Section 3 we update the derivations of short- and long-term marine water EQS, and Section 4 updates the sediment EQS. Section 5 discusses a recent field study commissioned by MSD, and Section 6 provides conclusions and recommendations based on the updated derivations.

2 NEW ECOTOXICITY DATA FOR EMAMECTIN BENZOATE

The following ecotoxicity tests with EMB were undertaken during 2017/2018, under the sponsorship of SSPO and MSD.

- Determination of acute toxicity of emamectin benzoate to lugworm (*Arenicola marina*) (10-day static) (EPP Environmental Centre 2018A).
- Determination of acute toxicity of emamectin benzoate to marine amphipods (*Corophium volutator*) (10-day static) (EPP Environmental Centre 2018B).
- Determination of acute toxicity of emamectin benzoate to mysid shrimp (*Americamysis bahia*) (96-hour static) (EPP Environmental Centre 2018C).
- Determination of chronic toxicity of emamectin benzoate to mysid shrimp (*Americamysis bahia*) (28-day flow-through) (EPP Environmental Centre 2018D).
- Emamectin benzoate: marine sediment chronic toxicity with amphipod (*Leptocheirus plumulosus*) (EPP Environmental Centre 2018E).
- Emamectin benzoate: A life-cycle toxicity test with the marine amphipod (*Leptocheirus plumulosus*) using spiked sediment (EAG Laboratories 2018).
- Emamectin benzoate: Determination of effects in a water-sediment system on growth and reproduction of *Corophium volutator* using spiked natural sediment (Scymaris Ltd 2018).

The final report for each of these studies, as issued by the laboratory conducting the study, has been evaluated for reliability and relevance for WFD EQS derivation according to the CRED approach recommended in the WFD EQS TGD. The outcome of these evaluations is summarised in the following sub-sections.

2.1 *Arenicola* 10-day sediment study

The design, results, and reliability/relevance assessment of the 10-day *Arenicola* marine sediment study conducted by the EPP Environmental Centre are shown in Table 2.1.

Table 2.1 *Arenicola* 10-day sediment study design, results, and reliability/relevance

Test type/guideline	Determination of acute toxicity of emamectin benzoate to lugworm (*Arenicola marina*) (10-day static)/ ICES TIMES No. 29
Test Matrix	Marine sediment (moisture content: 29.4%)
Test organisms	*Arenicola marina*
Nominal test concentrations	62.5, 125, 250, 500, and 1000 µg kg^{-1} dry weight
Mean measured test concentrations	19.9, 41.8, 110.8, 273.8, and 642.9 µg kg^{-1} dry weight
Exposure period	10 days
Numbers of test organisms per replicate	10
Number of replicates per concentration	1
Endpoints	Mortality and casting
Results (based on mean measured concentrations)	LC50 (mortality): 40.8 (95% confidence interval: 26.4 – 62) µg kg^{-1} dry weight (equalling 31.5 µg kg^{-1} wet weight) EC10 (casting): 12.9 (95% confidence interval: 3.6 – 21.6) µg kg^{-1} dry weight (equalling 10 µg kg^{-1} wet weight)
Reliability and relevance	Reliability: R2 – Reliable with restrictions Relevance: C1 – Relevant

2.2 *Corophium* 10-day sediment study

The design, results, and reliability/relevance assessment of the 10-day *Corophium* marine sediment study conducted by the EPP Environmental Centre are shown in Table 2.2.

Table 2.2 *Corophium* 10-day sediment study design, results, and reliability/relevance

Test type/guideline	Determination of acute toxicity of emamectin benzoate to marine amphipods (*Corophium volutator*) (10-day static)/ OSPAR 2005 Part B
Test Matrix	Marine sediment (moisture content: 26.7%)
Test organisms	*Corophium volutator*
Nominal test concentrations	25, 50, 100, 200, and 400 µg kg^{-1} dry weight
Mean measured test concentrations	12.9, 46.1, 99.4, 186.7, and 420 µg kg^{-1} dry weight
Exposure period	10 days
Numbers of test organisms per replicate	10
Number of replicates per concentration	2
Endpoints	Mortality
Results (based on mean measured concentrations)	LC50 (mortality): 141.5* µg kg^{-1} dry weight (equalling 111.7 µg kg^{-1} wet weight) NOEC (mortality): 99.4 µg kg^{-1} dry weight(equalling 78.5 µg kg^{-1} wet weight)
Reliability and relevance	Reliability: R2 – Reliable with restrictions Relevance: C1 – Relevant

* Confidence limits could not be calculated

2.3 *Americamysis* 96-hour marine water study

The design, results, and reliability/relevance assessment of the 96-hour *Americamysis* marine water study conducted by the EPP Environmental Centre are shown in Table 2.3.

Table 2.3 *Americamysis* 96-hour marine water study design, results, and reliability/relevance

Test type/guideline	Determination of acute toxicity of emamectin benzoate to mysid shrimp (*Americamysis bahia*) (96-hour static)/ OPPTS 850.1035 (1996)
Test Matrix	Sea water
Test organisms	*Americamysis bahia*
Nominal test concentrations	6.25, 12.5, 25, 50, and 100 ng L^{-1}
Measured test concentrations (calculated from measurements in stock solutions)	4.96, 10.05, 21.74, 34.01, and 93.74 ng L^{-1}
Exposure period	96 hours
Numbers of test organisms per replicate	5
Number of replicates per concentration	4
Endpoints	Mortality
Results (based on mean measured concentrations)	LC50 (mortality): 112.57 (95% confidence interval: 65.08 – 467.51) ng L^{-1} NOEC (mortality): 21.74 ng L^{-1}
Reliability and relevance	Reliability: R1 – Reliable Relevance: C1 – Relevant

2.4 *Americamysis* 28-day marine water study

The design, results, and reliability/relevance assessment of the 28-day *Americamysis* marine water study conducted by the EPP Environmental Centre are shown in Table 2.4.

Table 2.4 *Americamysis* 28-day marine water study design, results, and reliability/relevance

Test type/guideline	Determination of chronic toxicity of emamectin benzoate to mysid shrimp (*Americamysis bahia*) (28-day flow through)/ OPPTS 850.1350 (1996)
Test Matrix	Sea water
Test organisms	*Americamysis bahia*
Nominal test concentrations	2.5, 5, 10, 20, and 40 ng L^{-1}
Measured test concentrations (calculated from measurements in stock solutions)	2.02, 4.13, 7.84, 17.07 and 37.05 ng L^{-1}
Exposure period	28 days
Numbers of test organisms added per replicate	40 (as a single replicate (Day 0-14), then split between 4 chambers (Day 15-28)
Number of replicates per concentration	1 (1 tank containing one chamber each for F0 and F1; Day 0-14); (1 tank containing 1 F1 chamber, and 4 F0 chambers; Day 15-28)
Endpoints	Mortality (F0 & F1), reproduction, growth
Results (based on nominal concentrations)	EC10 (28-day mortality F0); 33.10 (95% confidence interval: 13.40 – 24585) ng L^{-1} NOEC (28-day mortality F0): 37.05 ng L^{-1} EC10 (28-day mortality F1): 24.52 (95% confidence limits: 15.97 – 52.73) ng L^{-1} NOEC (28-day mortality F1): 7.84 ng L^{-1} EC10 (Male growth): > 37.05 ng L^{-1} NOEC (Male growth): 37.05 ng L^{-1} EC10 (Female growth): > 37.05 ng L^{-1} NOEC (Female growth): 4.13 ng L^{-1}

	EC10 (reproduction): 9.44 (95% confidence limits: 1.72 – 15.01) ng L^{-1} NOEC (reproduction): 17.07 ng L^{-1}
Reliability and relevance	Reliability: R2 – Reliable with restrictions Relevance: C1 – Relevant

2.5 *Leptocheirus* 28-day sediment study (EPP)

The design, results, and reliability/relevance assessment of the 28-day *Leptocheirus* marine sediment study conducted by the EPP Environmental Centre are shown in Table 2.5.

Table 2.5 *Leptocheirus* 28-day sediment study design, results, and reliability/relevance (EPP)

Test type/guideline	Emamectin benzoate: A life-cycle toxicity test with the marine amphipod (*Leptocheirus plumulosus*) using spiked sediment/ EPA/600/R-01/020 (March 2001)
Test Matrix	Marine sediment (moisture content: 26.7%)
Test organisms	*Leptocheirus plumulosus*
Nominal test concentrations	31.25, 62.5, 125, 250, and 500 µg kg^{-1} dry weight
Mean measured test concentrations	Day 0: 21.7, 51.7, 90.6, 235.1, and 444.8 µg kg^{-1} dry weight Day 28: 8.6, 59.9, 84.6, 160.1, and 277.0 µg kg^{-1} dry weight
Exposure period	28 days
Numbers of test organisms per replicate	20
Number of replicates per concentration	4
Endpoints	Mortality, reproduction and growth
Results (based on mean measured concentrations)	LC10 (mortality): 22.9 (95% confidence interval: 18.1 – 27.2) µg kg^{-1} dry weight (equalling 18.1 µg kg^{-1} wet weight) EC10 (growth rate): 17.6 (95% confidence interval: 12.1 – 22.4) µg kg^{-1} dry weight (equalling 13.9 µg kg^{-1} wet weight) NOEC (reproduction): 51.7 µg kg^{-1} dry weight (equalling 40.8 µg kg^{-1} wet weight)
Reliability and relevance	Reliability: R2 – Reliable with restrictions Relevance: C1 – Relevant

2.6 *Leptocheirus* 28-day sediment study (EAG)

The design, results, and reliability/relevance assessment of the 28-day *Leptocheirus* marine sediment study conducted by EAG Laboratories are shown in Table 2.6.

Table 2.6 *Leptocheirus* 28-day sediment study design, results, and reliability/relevance (EAG)

Test type/guideline	Emamectin benzoate: marine sediment chronic toxicity with amphipod (*Leptocheirus plumulosus*)/ EPA/600/R-01/020 (March 2001)
Test Matrix	Marine sediment (moisture content: 26.4%)
Test organisms	*Leptocheirus plumulosus*
Nominal test concentrations	51, 130, 320, 800, and 2000 µg kg^{-1} dry weight
Mean measured test concentrations	38, 100, 260, 620, and 1600 µg kg^{-1} dry weight
Exposure period	28 days
Numbers of test organisms per replicate	20
Number of replicates per concentration	5
Endpoints	Mortality, reproduction and growth (male and female)
Results (based on mean measured concentrations)	LC10 (mortality): 75 (95% confidence interval: 38 – 150) µg kg^{-1} dry weight (59 µg kg^{-1} wet weight) EC10 (male growth rate): 57 (95% confidence interval: 18 – 180) µg kg^{-1} dry weight (46 µg kg^{-1} wet weight) EC10 (female growth rate): 49 (95% confidence interval: 11 – 210) µg kg^{-1} dry weight (39 µg kg^{-1} wet weight) EC10 (reproduction): 43 (95% confidence interval: 19 - 95) µg kg^{-1} dry weight (35 µg kg^{-1} wet weight) NOEC (reproduction): 38 µg kg^{-1} dry weight (30 µg kg^{-1} wet weight)

Reliability and relevance	Reliability: R1 – Reliable Relevance: C1 – Relevant

2.7 *Corophium* 75-day sediment study

The design, results, and reliability/relevance assessment of the 75-day *Corophium* marine sediment study conducted by Scymaris are shown in Table 2.7.

Table 2.7 *Corophium* 75-day sediment study design, results, and reliability/relevance

Test type/guideline	Emamectin benzoate: Determination of effects in a water-sediment system on growth and reproduction of *Corophium volutator* using spiked natural sediment/No guideline
Test Matrix	Marine sediment (moisture content: 65%)
Test organisms	*Corophium volutator*
Nominal test concentrations	0.02, 0.032, 0.1, 0.32, 1, 3.2, 10, and 32 µg kg^{-1} dry weight
Mean measured test concentrations	<LOD, <LOD, 0.02, 0.193, 0.861, 3.24, 10.4, and 61.3 µg kg^{-1} dry weight
Exposure period	75 days
Numbers of test organisms per replicate	20
Number of replicates per concentration	10
Endpoints	Mortality, Growth, Reproduction
Results (based on mean measured concentrations)	NOEC (mortality): > 61.3 µg kg^{-1} dry weight (equalling > 37.2 µg kg^{-1} wet weight) NOEC (growth): > 61.3 µg kg^{-1} dry weight (equalling > 37.2 µg kg^{-1} wet weight) NOEC (reproduction): > 61.3 µg kg^{-1} dry weight (equalling > 37.2 µg kg^{-1} wet weight)
Reliability and relevance	Reliability: R1 – Reliable Relevance: C1 – Relevant

3 EQS DERIVATION FOR MARINE WATER

3.1 Derivation of a short-term marine EQS (MAC-QS)

A short-term marine PNEC of 0.8 ng L^{-1} is proposed in the 2017 review of EQS for EMB (WRc 2017). This was derived using a deterministic approach in which an AF of 50 was applied to the lowest LC50 for species in the EMB acute marine dataset (0.04 µg L^{-1} from a 96-hour mortality test with the mysid shrimp *Americamysis bahia*). No reliable acute marine algal data were available for this substance, so it was proposed that (despite potential statistical differences in the acute ecotoxicity datasets for marine and fresh waters) the reliable freshwater algal data could be used to supplement gaps in the acute marine dataset. The AF was then applied in compliance with the WFD EQS TGD (EC 2011), which proposes that an AF of 50 is appropriate when a base-set of acute algal, invertebrate, and fish data are available, and an additional marine taxonomic group is represented in the marine dataset (in this case molluscs).

A statistical comparison of the short-term marine and freshwater datasets was undertaken in this previous assessment and highlighted a potential difference in the datasets, but potential issues were identified with the robustness of the statistical test owing to the small dataset size, differences in numbers of datapoints, the taxonomic representativeness of the datasets, and potential skewing of both datasets towards both the least and the most sensitive datapoints. The authors of that assessment suggested that, as crustaceans are likely to be the most sensitive taxonomic group in the marine environment, and algae appear to be much less sensitive, it was appropriate to use the freshwater algal data to fill the gap in the marine dataset.

In addition, a deterministic approach to EQS derivation was proposed by the authors of the previous assessment because there appeared to be insufficient data for a species sensitivity distribution (SSD) approach, which requires eight different taxonomic groups for compliance with the TGD. They suggested that only seven different taxonomic groups are available, although there actually appear to be only six taxonomic groups for the combined marine and freshwater dataset if bacteria are included and crustaceans are split into two groups (*Daphnia*, copepods, and shrimps separated from lobsters).

The 2018 testing programme for EMB summarised in Section 2 has generated only one new acute LC50 for marine species, and this is for a species that was already represented in the acute marine dataset in the previous EQS assessment (i.e. the mysid shrimp *A. bahia*). This study therefore adds nothing to the short-term ecotoxicity dataset in terms of taxonomic representativeness, and a probabilistic approach to MAC-QS derivation would not meet TGD requirements. It is still technically possible to construct an SSD using the combined marine and freshwater short-term datasets, and some regulatory jurisdictions apply this approach with much smaller datasets. An SSD approach would not be accepted by UK regulators if there is a requirement to follow the WFD EQS TGD (EC 2011). However, we have derived a probabilistic SSD for the EMB MAC-QS to provide a comparison with the deterministic approach.

For the deterministic approach we have incorporated the new acute mysid shrimp study (EPP 2018C) but have otherwise utilised the same dataset presented by the previous assessment (WRc 2017). Table 3.1 summarises the most sensitive acute marine data for the relevant taxonomic groups, except for algae for which no reliable short-term marine data are available.

Table 3.1 Most sensitive reliable short-term data for EMB by taxonomic group

Taxonomic Group	Species	LC/EC50 (µg L^{-1})	Study	Geometric mean (µg L^{-1})	Reliability Score
Algae (freshwater)	*Pseudokirchneriella subcapitata*	12.1	EFSA (2009) cited Maynard (2003a)*	9.33	1
		7.2	EFSA (2012), EC (2011b)*		2
Invertebrates	*Americamysis bahia*	0.04	WRc (2000)*	0.056	2
		0.04	US EPA (2009); EFSA (2009), Environment Canada (2005)*		2
		0.11	EPP 2018C		1
Fish	*Cyprinodon variegatus*	1430	WRc (2000)*	1430	2
		1430	Environment Canada (2005), EFSA (2009) cited 1995 data, EC (2011b)*		1
Additional marine group (molluscs)	*Crassostrea virginica*	490	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)*	573	2

Taxonomic Group	Species	LC/EC50 (µg L^{-1})	Study	Geometric mean (µg L^{-1})	Reliability Score
		670	Environment Canada (2005)*		2

* As cited in WRc (2017).

Table 3.1 shows that invertebrates (crustaceans) are clearly the most acutely sensitive taxonomic group, based on the available reliable acute ecotoxicity dataset.

The geometric mean of the three reliable LC50s for mortality of *Americamysis bahia* is 56 ng L^{-1}.

Using a deterministic approach and an AF of 50 (as applied in the previous assessment) results in a MAC-QS of 1.1 ng L^{-1}.

LC50 = 56 ng L^{-1} / AF = 50 = 1.1 ng L^{-1}

For the probabilistic approach, we have utilised all the available reliable, short-term marine and freshwater data for EMB. As with the deterministic assessment this comprises all the reliable short-term data presented in the previous assessment (WRc 2017), with the addition of the new acute mysid shrimp study (EPP 2018C).

Figure 3.1 shows the SSD distribution for the combined short-term marine and freshwater EMB dataset (n=16).

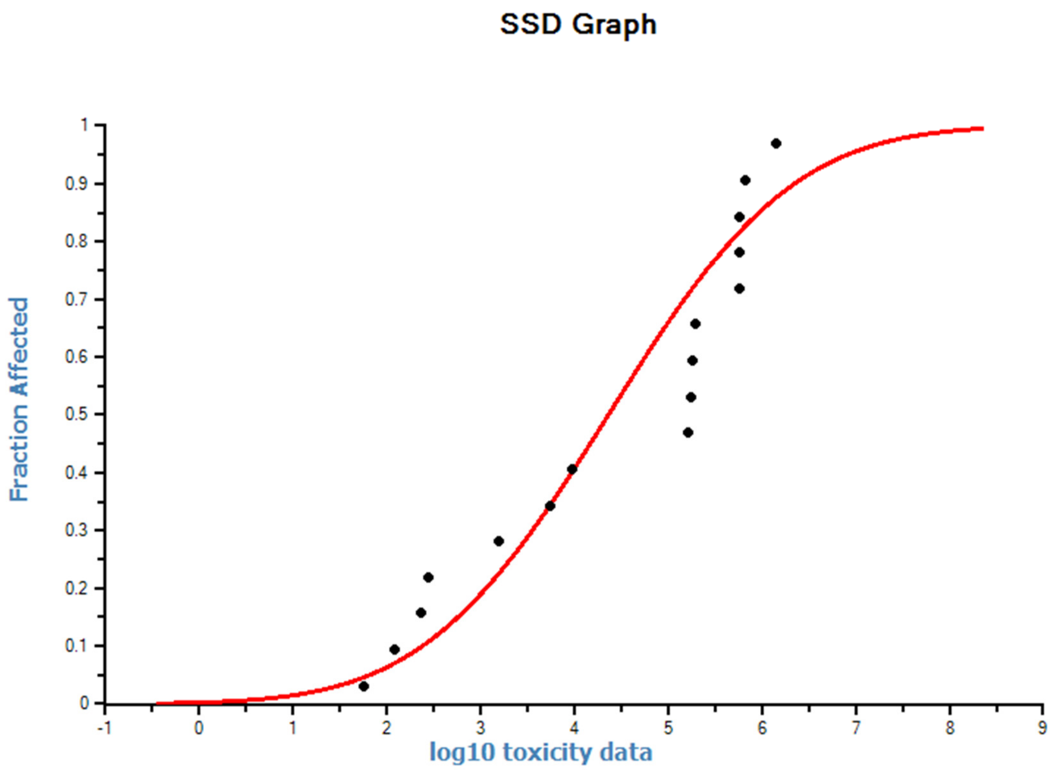

Figure 3.1 Species Sensitivity Distribution of acute data for EMB (combined marine and freshwater data (ng/L)) (n=16)[1]

The dataset used to construct the SSD in Figure 3.1 comprises only four taxonomic groups (marine crustaceans, marine bivalve molluscs, freshwater algae, and marine/ freshwater fish). The lowest five LC/EC50 values in the SSD are for crustaceans, and the lowest four are for benthic marine crustaceans.

Unfortunately, the SSD curve does not appear to fit a log-normal distribution and fails three statistical tests for normality at most significance levels (Table 3.2).

[1] Log-normal distribution from ETX. 2.1 (RIVM 2015)

Table 3.2 Goodness-of-fit statistics for SSD of acute data for EMB

Statistical test	Significance level	Normality
Anderson-darling	0.1	Rejected
	0.05	Rejected
	0.025	Rejected
	0.01	Accepted
Kolmogorov-Smirnov	0.1	Rejected
	0.05	Rejected
	0.025	Rejected
	0.01	Rejected
Cramer von Mises	0.01	Rejected
	0.05	Rejected
	0.025	Rejected
	0.01	Accepted

The HC5-50 (i.e. the Hazardous Concentration representing 50% or greater effect for 5% of the species) for EMB from the SSD in Figure 3.1 is 5.82 ng L^{-1}. The WFD EQS TGD (EC 2011) prescribes an AF of 10 to be applied to this HC5-50 to account for the extrapolation from EC/LC50 values to a 'no effect' threshold.

Using a probabilistic approach and an AF of 10 therefore results in a MAC-QS of 0.58 ng L^{-1}.

HC5-50 = 5.82 ng L^{-1} / AF = 10 = 0.58 ng L^{-1}

The probabilistic derivation of a MAC-QS for EMB is considered unreliable because of insufficient overall taxonomic coverage of the combined short-term marine and freshwater dataset, and the lack of a statistically significant fit to the log-normal distribution used to construct the SSD curve. Nevertheless, the approach visually illustrates that benthic marine crustaceans are clearly the most sensitive group to EMB.

3.2 Derivation of a long-term marine EQS (AA-QS)

A long-term marine EQS of 0.435 ng L^{-1} is proposed in the 2017 review of EQS for EMB (WRc 2017). This was derived using a deterministic approach in which an AF of 20 was applied to the lowest NOEC for species in the EMB chronic marine dataset (NOEC for growth of 8.7 ng L^{-1}, from a 28-day reproduction test with the mysid shrimp *A. bahia*). The AF was selected on the basis that the reliable chronic dataset for EMB contains three freshwater taxonomic groups (fish, invertebrates, and algae) and an additional marine group (marine bivalve molluscs) (*A. bahia* is not considered to be an 'additional marine group' according to the WFD EQS TGD (EC 2011)).

The 2018 testing programme for EMB summarised in Section 2 has generated only one new chronic study for marine species, and this is for a species that was already represented in the chronic marine dataset in the previous EQS assessment (i.e. the mysid shrimp *A. bahia*). This study therefore adds nothing to the long-term ecotoxicity dataset in terms of taxonomic representativeness, and a probabilistic approach to AA-QS derivation would not be compliant with the TGD.

For the deterministic approach detailed below we have incorporated the new chronic mysid shrimp study (EPP 2018D) but have otherwise utilised the same dataset as presented by the previous assessment (WRc 2017). Table 3.3 summarises the most sensitive chronic data for the relevant taxonomic groups.

Table 3.3 Most sensitive reliable long-term data for EMB by taxonomic group

Taxonomic Group	Species	EC10/ NOEC (ng L^{-1})	Study	Geometric mean (ng L^{-1})	Reliability Score
Algae (freshwater)	*Pseudokirchneriella subcapitata*	< 3900	EFSA (2012), US EPA (2009); ECOTOX (2016) cited US Pesticide Ecotoxicity Database (1992) *	Not applicable	1
Invertebrates	*Americamysis bahia (28-day growth)*	8.7	US EPA (2009), ECOTOX (2016) *	5.99	2
		4.13 (females only)	EPP 2018D		2
	Americamysis bahia (reproduction)	9.44	EPP 2018D	Not applicable	2
Fish (freshwater)	*Pimephales promelas*	6500	US EPA (2009) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992) *	Not applicable	2
Additional marine group (molluscs)	*Crassostrea virginica*	260000	WRc (2000) *	Not applicable	2

* As cited in WRc (2017)

Invertebrates (crustaceans) are clearly the most chronically sensitive taxonomic group, based on the available reliable chronic ecotoxicity dataset. In addition to the data for *A. bahia* shown in Table 3.3, the marine crustacean dataset also contains data for reproduction of the copepod *Acartia clausi*, giving a NOEC of 50 ng L^{-1}.

In the previous assessment (WRc 2017) a NOEC for *A. bahia* growth (8.7 ng L^{-1}) was applied as the driving datapoint for the long-term marine EQS. The new *A. bahia* study (EPP 2018D) also measured growth in both male and female mysids over the 28-day exposure period. However, the results in this study are equivocal, with a NOEC for female growth of 4.13 ng L^{-1}, but an EC10 for the same measurement of > 37.05 ng L^{-1} (the highest exposure concentration) being reported. For male growth in the same study both the NOEC and the EC10 are reported as > 37.05 ng L^{-1}. The apparent effect on female growth of mysids at exposure concentrations of 7.84 ng L^{-1} and above, while statistically significantly different from controls and dose-dependent, is slight (i.e. <10%) as evidenced by the inability to derive an EC10 for the same endpoint. It also seems unlikely that EMB could cause differential growth effects on male and female organisms.

The geometric mean of the two reliable NOECs for *Americamysis bahia* growth is 5.99 ng L^{-1}, while the EC10 for reproduction (EPP 2018D) is 9.44 ng L^{-1}.

Using a deterministic approach and an AF of 20 (as applied in the previous assessment) results in AA-QS of 0.3 and 0.47 ng L^{-1}, based on growth and reproduction effects in *A. bahia* respectively.

NOEC/ EC10 (growth) = 5.99 ng L^{-1} / AF = 20 = 0.3 ng L^{-1}

NOEC/ EC10 (reproduction) = 9.44 ng L^{-1} / AF = 20 = 0.47 ng L^{-1}

Given the proximity of the two values, and uncertainty about the reliability of the growth endpoint from the *A. bahia* tests, we recommend that the marine AA-EQS of 0.47 ng L^{-1} is taken forward. This value is similar to the EQS derived in the previous assessment (0.435 ng L^{-1}) (WRc 2017).

4 EQS DERIVATION FOR SEDIMENT

As noted in the introduction to this report, no long-term ecotoxicity effect data (e.g. growth or reproduction) were available for marine sediment organisms at the time of the previous EQS development (WRc 2017). That review therefore proposed 'far-field' and 'near field' marine sediment EQS for EMB based on a single long-term sediment study undertaken with the freshwater insect *Chironomus riparius.*

The EQS values were derived using the NOEC for the emergence of adult midges from pupae (1.175 µg kg^{-1} dry weight), to which AFs of 10 and 100 were applied to give proposed 'far field' and 'near field' EQS of 120 and 12 ng kg^{-1} dry weight, respectively.

The 2018 testing programme was primarily designed to address the gap in the long-term sediment ecotoxicity dataset for EMB with respect to marine organisms. Two short-term (10-day) marine sediment studies were conducted with the lugworm *Arenicola marina* and the crustacean amphipod *Corophium volutator* (EPP 2018A, 2018B), and three long-term (28-75 day) studies were conducted with marine crustaceans (two with *Leptocheirus plumulosus* and one with *Corophium volutator*) (EPP 2018E, EAG 2018, Scymaris 2018). As summarised in Section 2, all five of these studies are reliable and relevant for EQS derivation.

The results of these five studies are summarised in Table 4.1.

Table 4.1 Summary of sediment toxicity tests undertaken in 2018 for EMB

Species	Endpoint	Threshold	Value (µg/kg dry weight)	Reference
Arenicola marina	10-day mortality	LC50	40.8	EPP 2018A
	10-day casting	EC10	12.9	
Corophium volutator	10-day mortality	LC50	142	EPP 2018B
	75-day mortality	NOEC	> 61.3	Scymaris 2018
	75-day reproduction	NOEC	> 61.3	
	75-day growth	NOEC	> 61.3	
Leptocheirus plumulosus	28-day mortality	LC10	22.9	EPP 2018E
		LC10	75	EAG 2018
		Geometric mean of LC10 values	41.4	EPP 2018E EAG 2018
	28-day growth	EC10	17.6	EPP 2018E
		EC10	49 and 57	EAG 2018
		Geometric mean of EC10 values	36.6	EPP 2018E EAG 2018
	28-day reproduction	NOEC	51.7	EPP 2018E
		EC10	43	EAG 2018
		Geometric mean of EC10 and NOEC values	47.2	EPP 2018E EAG 2018

The data derived for marine species significantly expands the available reliable data for EMB ecotoxicity to benthic organisms and they are sufficient to derive a marine sediment EQS without the need to include the freshwater (*C. riparius*) data.

The larvae of *C. riparius* live and feed in freshwater sediments, but adults are not aquatic. In addition, the most sensitive endpoint in the *C. riparius* study was adult emergence from pupae (i.e. following metamorphosis from larvae). There are no truly marine insect species. From the 25,000-30,000 insect species that are aquatic or have aquatic larval stages, only a fraction, perhaps several hundred species, are marine or intertidal (Cheng 1976). Their habitat is limited to transitional environments provided by estuaries, saltmarshes, mangrove swamps, and the intertidal zones (Cheng 1976). Furthermore, since there are no marine invertebrate species which have life cycles involving aquatic larvae and non-aquatic adults, this study could be considered as not relevant for the derivation of a long-term marine sediment EQS for EMB.

We have therefore derived a sediment EQS for EMB using only marine sediment data. The lowest 'no effect' value is an EC10 for casting from the 10-day *Arenicola* study. An AF of 10 can be applied because the dataset covers three long-term *marine* sediment studies (*Arenicola*, *Leptocheirus*, and *Corophium*), resulting in a marine sediment EQS of 1.29 µg kg^{-1} dry weight (1290 ng kg^{-1} dry weight; 997 ng kg^{-1} wet weight).

A 'near field' marine sediment EQS was also derived in the previous EQS assessment (WRc 2017) in which an AF of 10 was applied to the only sediment NOEC available at that time. It is not clear if SEPA currently still implements 'near field' EQS for salmon farming activity in Scotland. The precise approach that should be applied in the derivation of 'near field' sediment EQS (which are not applied under the WFD) is also unclear. The previous assessment (WRc 2017) simply reduced the 'far field' AF by a factor of 10 (i.e. from 100 to 10) to derive the 'near field' EQS. However, we have already applied an AF of 10 to derive the 'far field' EQS, and therefore reduction of the AF by a factor of 10 would result in no AF being applied to the relevant NOEC or EC10. It would therefore seem sensible to reduce the AF by a lower factor (e.g. 2 or 5) to extrapolate from a 'far field' to 'near field' EQS. An AF of 5 (x 2 reduction) would result in a 'near field' EQS of 2580 ng kg^{-1} dry weight, while reduction of the AF by 5 (AF=2) would result in a 'near field' EQS of 6450 ng kg^{-1} dry weight.

These EQS are environmentally responsible values, derived according to the relevant WFD guidance (EC 2011) and supported by reliable and sensitive laboratory ecotoxicity data.

However, the *Arenicola* casting endpoint is derived from an exposure of relatively short duration (10 days) and could be considered to be unrepresentative of the long-term toxicity of EMB to polychaetes.

An additional full chronic ecotoxicity study on EMB in benthic marine species has therefore been commissioned by MSD to address this potential data gap. This ASTM guideline E1611 (Standard Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids) study will be carried out at Scymaris laboratories in Brixham and will be GLP compliant. The test species will be the polychaete worm, *Hediste diversicolor* (the European ragworm). The exposure period will be 28 days and growth will be assessed as the chronic endpoint. The experimental phase of the study will commence in January 2019 with a range-finder test, and the main test will start in March 2019. The draft report is expected in June 2019.

4.1 Estimation of a sediment EQS by equilibrium partitioning

It is also possible to estimate a sediment EQS using long-term water column toxicity data and equilibrium partitioning. In order to support the sediment EQS derived above we have also estimated a sediment EQS based on equilibrium partitioning using the approach given in the WFD EQS TGD (EC 2011).

Table 4.2 shows the EMB and sediment characteristics, and ecotoxicity values applied in the equilibrium partitioning estimate of the sediment EQS for EMB.

Table 4.2 Parameters applied in sediment equilibrium partitioning estimate for EMB

Parameter	Value
Fraction organic Carbon (FOC_{sed})	Standard TGD sediment: 0.05 Sediment used in Scymaris 2018: 0.0575
Partition coefficient between organic carbon and water (K_{oc}) for EMB	Lowest value (clay loam) (WRc 2017): 25363 L kg^{-1} Highest value (WRc 2017): 728918 L kg^{-1} Average value (WRc 2017): 265687 L kg^{-1}
Fraction air in sediment ($Fair_{sed}$)	0 (Default)
Fraction water in sediment ($Fwater_{sed}$)	0.8 (Default)
Fraction solids in sediment ($Fsolid_{sed}$)	0.2 (Default)
Density of solid phase (RHO_{solid})	2500 kgww m^{-3}
Bulk density of wet sediment (RHO_{sed})	1300 kgsolid $msolid^{-3}$
Conversion factor for sediment concentration wet-dry weight sediment	2.6 $kgww.kgdw^{-1}$
Ecotoxicity value (28-day reproduction for *A. bahia*)	9.44 ng L^{-1}

The equilibrium partitioning sediment EQS has been estimated for both the standardised TGD sediment and the sediment used in the new long-term *Corophium* test (Scymaris 2018) as follows:

1. Organic carbon content for standardised TGD sediment and lowest, highest, and mean sediment partitioning for EMB, and

2. Organic carbon content for Scymaris (2018) sediment and lowest, highest, and mean sediment partitioning for EMB.

The Scymaris (2018) sediment has been used to represent the characteristics of the sediment likely to be found in the vicinity of salmon pens since it is a muddy sediment, with an organic carbon content closest to the standardised TGD sediment. The sediments applied in the other sediment tests carried out in 2018 all featured sandy sediments with low organic carbon contents (0.2 to 0.3%).

Table 4.3 shows the equilibrium partitioning sediment EQS estimated using the parameters in Table 4.2.

Table 4.3 Estimated equilibrium partitioning sediment EQS for EMB

Sediment	EMB K_{oc}	Partition coefficient solid-water in sediment (Kp_{sed})	Partition coefficient between sediment and water ($K_{sed\text{-}water}$)	$QS_{sediment,EqP,ww}$ (ng kg^{-1} wet weight)	$QS_{sediment,EqP,dw}$ (ng kg^{-1} dry weight)
Standardised TGD sediment	25363 L kg^{-1}	1268	635	461	1199
	728918 L kg^{-1}	36446	18224	13233	34406
	265687 L kg^{-1}	13284	6643	4824	12541
Scymaris (2018) sediment	25363 L kg^{-1}	1458	730	530	1378
	728918 L kg^{-1}	41913	20957	15218	39567
	265687 L kg^{-1}	15277	7639	5547	14423

The worst-case estimated equilibrium partitioning sediment EQS for EMB from Table 4.3 is 1199 ng kg^{-1} dry weight (1.2 µg kg^{-1} dry weight). This compares well with the sediment EQS derived from experimental studies.

5 FIELD DATA

SAMS was contracted by MSD to undertake a research programme to investigate the dispersal and fate of EMB and potential effects on marine benthic crustaceans following its use in salmon aquaculture in Scotland. Due to the properties of EMB (e.g. low water solubility and high adsorption potential to particles), it will be present in the sediment rather than the water column.

One element of the research programme was a Passive Field Monitoring Survey. The objective of the survey was to compare EMB concentrations present in sediment from different water bodies with different histories of EMB use. The study involved a large-scale sediment sampling survey programme undertaken between July – October 2017, which ranged over a broad geographical scale. The sampling programme targeted 21 fish farm sites from three areas in Scotland: Shetland/Orkney, the Western Isles, and the West Coast.

At each site sediment samples were obtained from different stations and sub-sampled for various parameters: Total Organic Carbon (TOC), Particle Size Analysis (PSA), and EMB Chemical Residue (CR), combined with the identification and enumeration of the benthic infaunal community present in each sample. The sub-samples were analysed to determine whether there was a widespread effect on the infaunal and, in particular, the crustacean community living in or on the sediment.

5.1 Passive field monitoring survey methodology

A stratified random sampling programme was designed to collect samples from fin fish aquaculture sites across Scotland[2]. Aquaculture sites were first stratified into three broad geographical regions. These regions were:

- Shetland and Orkney;
- Western Isles (Outer Hebrides); and
- West Coast (sites located around the Inner Hebrides and on the West Coast of Scotland).

Once sites had been allocated into each of these geographical areas a further level of stratification was applied according to the EMB use at each site, calculated from EMB (g) use data as presented on the SEPA database[7]. EMB use over the previous three years was considered (2014-2016, where available), and a mean level of use was calculated. Each site was assigned a "use level" based on the following criteria:

- Low: <100 kg EMB used per year;
- Medium: 100-200 kg EMB used per year; or

[2] Based on the Scottish Environment Protection Agency (SEPA) database on fin fish aquaculture (https://www.environment.gov.scot/data-analysis-applications/marine-fish-farm/)

- High: >200 kg EMB used per year.

Farm sites were then selected at random from each sub-division of geographical area and historic EMB use. Seven sites were selected from each of the three geographical regions, with two categorised as high EMB use, three as medium EMB use, and two as low EMB use. At the end of the process, 21 sites had been selected.

The selected sites represented different hydrodynamic conditions and bathymetry. The current velocity, and thus the potential for resuspension and dispersion of sediment and sediment-associated EMB, varied as shown by the distance of the calculated Far Field Maximum Accepted Concentration (FFMAC) stations, which ranged from 73 – 178 m in the downstream direction and 35 – 144 m in the upstream direction.

At each of the 21 sites, ten sampling locations (stations) were identified. The stations were distributed along a rough transect across each fish farm site. The sampling stations were selected using AUTO DEPOMOD output to identify the Near-Field-Zone (NFZ) (EQS for EMB residues of 7.63 µg/kg of sediment wet weight), as well as the FFMAC (with an EQS for EMB residues of 0.76 µg/kg of sediment wet weight). FFMAC distance was defined as the maximum distance from zone to cage and was site-specific. The ten sampling stations at each site were selected and numbered according to the following scheme:

1. Cage Edge (downstream)
2. NFZ boundary (downstream)
3. Reference station (downstream, as provided by the operator from benthic monitoring)
4. FFMAC (upstream)
5. FFMAC (side) distance x 4 whichever side is feasible - Proposed Negative Control (Near Field)
6. FFMAC distance (downstream)
7. FFMAC distance x 4 (downstream)
8. FFMAC distance x 15 (downstream)
9. FFMAC distance x 25 or a minimum of double the reference station distance whichever is furthest (up to 3 km max).
10. FFMAC distance x 8 (upstream) - Proposed Negative Control (Far Field)

The survey design aimed to provide a gradient of sampling away from the cages to a minimum distance of 2 km. One station at each site (#3) was the designated reference station (the statutory benthic reference), and each site included a minimum of two stations sampled at a distance beyond this reference station, one of which was a minimum of double the distance from cage edge to reference, up to a maximum of 3 km.

Once in the field, the exact number of samples that could be taken depended on the weather, sea state, and sediment conditions. Stations were sampled from "negative control" stations first, then in decreasing distance to the fish farm, with the "cage edge" sampled last, to minimise the risk of cross-contamination.

Of the 21 planned survey sites, 19 were successfully sampled. The sites were labelled in alphabetical order: Site A to Site S. Poor weather during September and October 2017 meant that suitable survey windows could not be found to undertake the sampling at all sites. A total of 180 stations was successfully sampled at these 19 sites. Unsuccessful samples were due to a variety of reasons, including the seabed being unsuitable for grab sampling (i.e. the sediment was too hard), vessel equipment failure, and poor weather conditions. Some pooling of samples was undertaken when frequent low-volume samples were obtained from one location. In these samples, physicochemical samples (i.e. PSA, TOC, and EMB CR) were taken from one grab, with the remaining volume added to subsequent grab(s) to provide a sample of sufficient size for the faunal analysis.

Grab sampling was carried out using a 0.1 m^2 van Veen grab. Four sub-samples were collected for EMB CR analysis from each grab sample. After the EMB CR samples had been taken, PSA and TOC samples were collected. After the physicochemical sub-samples had been acquired the remaining sample was retained for faunal analysis.

EMB CR analysis was undertaken by Charles River Laboratories (CRL) according to Good Laboratory Practice (GLP). Faunal analysis was undertaken by Thomson Ecology Ltd. Faunal samples were provided to the laboratory fixed in formalin and were sorted under low-powered light microscopes to remove any fauna, which were then passed on to expert taxonomists for identification and enumeration.

5.2 Summary of results from passive field monitoring survey

EMB concentrations were highest at stations close to fish farm cages and declined with increasing distance from the fish farms.

The current EQS for EMB as recommended by SEPA is a MAC of 0.763 µg kg^{-1} wet weight outside a zone of effects area, which is defined as >100 m from the edge of fish farm cages. Of the 508 replicates from 127 stations located beyond 100 m from the cage edge, only 9 replicates had EMB concentrations higher than the EQS. These replicates came from three stations at three different sites: Site_A_006 (2 out of 4 replicates), Site_K_006 (3 out of 4 replicates), and Site_R_002 (all 4 replicates). Mean EMB concentration values showed that only two stations beyond 100 m from the cage edge exceeded the EQS for EMB. When the 100 m zone of effect is adjusted to account for current flow at each site, 436 replicates from 109 stations were located beyond the

FFMAC distance from the cage edge across all sites. None of these replicates exceeded the EQS for EMB, and no station had a mean EMB concentration exceeding the EQS.

There was not much difference in EMB concentrations in samples from upstream and downstream sampling stations. Most sites were in tidal areas, so this was not a surprising result, with currents flowing in both directions to disperse particles equally both upstream and downstream. The sampling stations located at FFMAC x4 distance at a tangent to flow at fish farm sites tended to have comparable EMB concentrations to samples from at least FFMAC x8 distance, showing that dispersion of particles is driven by the predominant current flow.

Although the highest EMB concentrations were measured at the Cage Edge stations, the NFZ Boundary stations had the highest minimum EMB values. This suggests that at some sites particles can be quickly dispersed away from the immediate footprint of the fish farm cages, settling at the NFZ rather than immediately below the source of input.

EMB concentrations recorded from the FFMAC x25 distance stations were not significantly different from the cage edge stations at Low SLICE® use sites, although concentrations from FFMAC x8 upstream and FFMAC x15 downstream stations were lower. This suggests that some of the samples for the FFMAC x25 downstream stations may have been influenced by EMB sources other than the sites surveyed (i.e. other nearby fish farm sites).

Whilst the EMB dispersal patterns around the fish farm sites showed clear patterns, the survey programme was not able to identify similarly clear patterns in the benthic crustacean community. Benthic crustacean species richness was lower closer to fish farm cages, although not to a significant degree. This was mirrored by the faunal community as a whole, indicating impacts on the entire benthic community for fish farm operations. The influence of site-specific environmental factors on the benthic crustacean (and total faunal) community was seen in the high degree of variability in faunal metrics derived from the raw enumerated and identified faunal samples. Due to this background environmental variability, it was not possible to quantify accurately the effect of EMB on the benthic crustacean community. There appeared to be a negative relationship between EMB and crustacean species richness, with species richness decreasing as EMB increased. However, the variability of the data set and the relative scarcity of sediment samples where EMB residues were measured at concentrations >0.5 µg/kg wet weight meant that this was only a weak negative relationship. Truncating the data to remove EMB concentrations <0.01 µg kg^{-1} wet weight and >1 µg kg^{-1} wet weight showed no relationship between mean EMB concentration and crustacean species richness. It is not possible to derive a No Effects Limit for EMB on macro-benthic crustaceans from the current data set.

The data set demonstrated that certain environmental factors appeared to have a higher importance in controlling the structure of benthic marine crustacean communities. Two, in particular, were examined: TOC and sediment particle size. The strongest relationship was between sediment particle size and crustacean species richness, with species richness increasing as overall particle size increased. EMB concentrations were negatively related to the mean particle size, albeit weakly. Therefore, those stations with higher EMB concentrations were also more likely to have finer sediment, and thus poorer crustacean communities even before any potential influence of EMB. If high EMB concentrations are more likely found where crustacean communities are sparse due to the underlying environmental conditions, detecting any additional impact from EMB would be unlikely.

Overall, the data show that the species richness of the benthic crustacean community at the surveyed sites is structured principally by the particle size of the sediment. Negative relationships were found between crustacean species richness and TOC and EMB concentrations, but the underlying variability in the data set meant that these trends were only weak. It is also important to note that other environmental factors, such as depth, are likely to influence community structure, but these have not been taken into consideration during the assessments in this report.

5.3 Conclusions from passive field monitoring survey

The passive field monitoring survey showed that the crustacean data are very noisy and that a linear regression through these and the EMB concentration data is influenced by data at the extremes of the distribution. As a result of this there was no apparent relationship between EMB concentration and crustacean richness when the data were truncated and data at very low EMB concentrations were removed.

There is a large discrepancy between the results of the recent long-term sediment toxicity tests (EPP 2018E, EAG 2018, Scymaris 2018) and apparent field effects on crustacean richness which are associated with very low EMB concentrations. There were limited effects on the most sensitive measured *L. plumulosus* endpoint (growth) at an EMB concentration of 17600 ng kg^{-1} dry weight (corresponding to 13891 ng kg^{-1} wet weight). However, in the field study there was an association between a decline in crustacean richness and EMB concentrations from above the LOQ of 1.5 ng kg^{-1} wet weight to approximately 50 ng kg^{-1} wet weight, after which there was no further decline in crustacean richness.

This enormous difference between laboratory and field results can be explained by the following features of the field survey data:

- Sixty-seven percent of the crustacean taxa present in samples in which the EMB concentration was <LOQ, but not present in samples in which the EMB concentration was between the LOQ and 50 ng kg^{-1} dry weight, occurred only once or twice across the 16 <LOQ samples. This lack of both abundance and taxonomic consistency across samples with EMB concentrations <LOQ suggests that other environmental factors were responsible for the pattern of distribution.

- When more than ten individuals from a taxon were found only in samples with EMB concentrations <LOQ then these were overwhelmingly from either Site S,

or from Site F sample 05, and not at other sites. This suggests that other environmental factors at Site S, and at Site F sampling point 05, were responsible for the pattern of distribution.

- Six taxa (Pycnogonida, *Melphidippella macra*, *Gnathia*, *Tryphosa nana*, *Cheirocratus sundevalli*, and Caprellidae) were found in at least one sample with EMB concentrations <LOQ and also in at least one sample with EMB concentrations >50 ng kg^{-1} dry weight, but not in samples at intermediate concentrations. This lack of a concentration-response suggests that the presence or absence of these taxa could not have been directly related to EMB concentration.

The field study therefore provides rather limited information for use in setting an EQS for EMB because there is no concentration-response relationship between EMB and crustacean richness above a value from approximately 50 ng kg^{-1} wet weight up to 1000 ng kg^{-1} wet weight. The apparent decline in crustacean richness between the LOQ and 50 ng kg^{-1} wet weight is toxicologically implausible given what we know of EMB's mode of action and appears to be driven by site-specific environmental factors other than EMB concentration. It is also toxicologically implausible to find what is in effect a "reverse threshold". One would normally expect to find that a toxic substance has negligible effects across a range from zero exposure up to a threshold at which effects begin to manifest themselves. These effects then increase with exposure concentration. In contrast with this concentration-response model, the data show an immediate decline in crustacean richness at very low EMB concentrations until a threshold is reached, again at a low concentration, beyond which increasing concentrations of EMB appear to have no additional effect. EMB toxicity is not a plausible mechanism for this at the concentrations reported by SAMS.

Nevertheless, the data generated in the field study support the proposed 'far field' sediment EQS of 1290 ng kg^{-1} dry weight (corresponding to 997 ng kg^{-1} wet weight). This EQS lies in the concentration range where no concentration-response relationship between EMB and crustacean richness occurs. Accordingly, a toxic effect at this concentration can be excluded.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations can be drawn from the information presented in this report.

- An extensive programme of new marine ecotoxicity testing on EMB has been completed successfully. This comprises two new studies on EMB ecotoxicity to a pelagic marine crustacean, and five new studies on the ecotoxicity of EMB to benthic marine organisms (two crustaceans and one polychaete).
- All of the new studies were conducted according to the principles of GLP, within reputable Contract Research Organisations, and to internationally standardised guidelines (or accepted peer-reviewed methodologies where standardised guidelines are not available).
- All seven of the new ecotoxicity studies on EMB should be considered to the reliable and relevant for EQS derivation, according to the CRED reliability and relevance assessment approach (as recommended by the WFD EQS TGD).
- Significant gaps in the long-term sediment ecotoxicity dataset for EMB have been addressed, such that the reliable and relevant dataset now includes four studies undertaken with benthic marine species.
- The new ecotoxicity data have been used to update the EQS for sediment and water proposed by WRc (2017).
- We propose a short-term marine water EQS (MAC-QS) of 1.1 ng EMB L^{-1}, based on a geometric mean LC50 (n=3) of 56 ng EMB L^{-1} for the mysid shrimp (*A. bahia*) and an Assessment Factor of 50. This represents a slight increase from the MAC-QS proposed by WRc (2017) of 0.8 ng EMB L^{-1}.
- We propose a long-term marine water EQS (AA-QS) of 0.47 ng EMB L^{-1}, based on an EC10 for reproduction of 9.44 ng EMB L^{-1} for the mysid shrimp (*A. bahia*) and an Assessment Factor of 20. This represents a slight increase from the MAC-QS proposed by WRc (2017) of 0.435 ng EMB L^{-1}.
- We propose a marine sediment EQS ('far field' sediment EQS) of 1290 ng EMB kg^{-1} dry weight (corresponding to 997 ng kg-1 wet weight), based on an EC10 for casting of 12900 ng EMB kg^{-1} dry weight for the lugworm (*Arenicola marina*) and an Assessment Factor of 10. The proposed sediment EQS is higher than that currently implemented by SEPA (763 ng kg^{-1} wet weight). The higher 'far field' sediment EQS derived here results from a dataset comprising studies that are highly relevant for the assessment of the potential hazards of EMB to sensitive groups of marine organisms, and an associated reduction in the AF applied. The currently implemented 'far field' EQS was based on a limited dataset of acute toxicity data, with a necessarily higher AF. The 'far field'

sediment EQS proposed here also represents a significant increase from the 'far field' sediment EQS proposed by WRc (2017) of 12 ng EMB kg^{-1} dry weight.

- The proposed 'far field' sediment EQS may be subject of revision after completion of the full chronic ecotoxicity study with the European ragworm that was commissioned by MSD. A draft report for this study is expected in June 2019.

- The proposed 'far field' sediment EQS is derived solely from ecotoxicity data on marine benthic species (four studies), of which two of the three tested species are benthic crustaceans, and the other a benthic polychaete. According to the results of the new laboratory studies, the sensitivity of marine benthic species to EMB is considerably lower than for freshwater species (based on a single freshwater sediment study with insect larvae).

- EMB acts as a chloride channel activator in arthropods, thus exerting a selective toxicity against sea lice. In the marine environment, this mode of action means that crustaceans are likely amongst the most sensitive taxonomic groups to EMB. Macrocyclic lactones, such as EMB, primarily act by binding to glutamate-gated chloride channels, which were first described in insects and crustacea (Wolstenholme 2012). Similar receptors and channels are expressed on neurons and muscle across different protostome phyla (Wolstenholme 2012). Accordingly, a high sensitivity can also be assumed for polychaetes, which belong to the phylum Annelida.

- The long-term marine sediment dataset now contains reliable and relevant data for two benthic crustacean species (across three studies), and a benthic polychaete, thus representing taxonomic groups of high sensitivity. A comparison of data for insects and crustaceans shows that the sensitivity of benthic crustaceans to sediment-bound EMB is much lower than the EQS proposed by SEPA (12 ng kg^{-1}). The most sensitive chronic endpoint for crustaceans (*L. plumulosus* growth) is 17600 ng kg^{-1} dry weight, corresponding to a single-study EQS of 176 ng kg^{-1} dry weight (when applying the same AF of 100 as for the *C. riparius* study).

- As there are now sufficient reliable long-term ecotoxicity data for marine benthic organisms, especially when the full chronic ecotoxicity study with the European ragworm is completed, we recommend that the marine sediment EQS is derived solely using marine species. The rejection of the *Chironomus* study is thereby in line with the WFD EQS TGD, which states that pooling of freshwater and saltwater data is only acceptable if differences in sensitivity between freshwater and saltwater organisms are not apparent.

- We propose that the Assessment Factor is set at the minimum recommended by the WFD EQS TGD (i.e. 10) on the basis that the TGD states "…uncertainty is reduced when there are relevant test endpoints from ecotoxicity studies that are highly relevant to a substance's mode of toxic action. An example would be fish life cycle studies for a chemical that is known to affect the reproductive physiology of vertebrates. Similarly, if a substance has a specific mode of toxic action, and reliable data for taxa that would be expected to be particularly sensitive are available (e.g. data for a range of insects for an insecticide that acts by inhibiting acetyl cholinesterase activity, or data for blue-green algae when dealing with chemicals that have bactericidal properties) then, again, an important aspect of uncertainty is reduced. Under these conditions, a smaller AF than the default value may be justified." EMB clearly falls into this class of substances with a specific mode of toxic action.

- We also recommend that if a 'near field' sediment EQS is required an AF of 5 is applied to the same long-term marine ecotoxicity dataset to give a 'near field' EQS of 2580 ng kg^{-1} dry weight. The selection of this AF is a balance between the assumed requirement for a higher 'near field' than 'far field' EQS, and the available options for a reduced AF (ranging from 1-5).

- The EQS recommended here are proposed as environmentally responsible values, supported by reliable and sensitive laboratory ecotoxicity data. Further support for the sediment EQS is provided by the outcome of an extensive field monitoring study.

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