Review of Environmental Quality Standard for Emamectin Benzoate

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Glossary

AC50	Half-maximal Activity Concentration
BCF	Bioconcentration factor
bw	Body weight
CAS	Chemical Abstracts Service
EC50	Concentration effective against 50% of the organisms tested
EMB	Emamectin benzoate
EQS	Environmental Quality Standard
GC	Gas chromatography
GC-MS	Gas chromatography/mass spectrometry
GLP	Good Laboratory Practice (OECD)
LC50	Concentration lethal to 50% of the organisms tested
LOAEL	Lowest observed adverse effect level
LOEC	Lowest observed effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
SEPA	Scottish Environment Protection Agency
US EPA	US Environmental Protection Agency

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Summary

i Project Aim

In 1999, the Scottish Environment Protection Agency (SEPA) undertook a risk assessment of emamectin benzoate in the marine environment following its administration to marine cage fish for control of sea lice infestations. As part of this risk assessment, SEPA derived Environmental Quality Standards (EQS) for the sea water and sediment. The EQS included a "far-field" sediment Maximum Acceptable Concentration (MAC) for the protection of all marine life >25 m from the marine cages and a "near-field" MAC trigger value for additional monitoring applicable to sediment within 25 m of the marine cages. An EQS-MAC for the water column was also derived. It has been 16 years since the data on emamectin benzoate has been reviewed and the aim of this project was to review all of the current data to determine if the EQSs derived in 1999 remained scientifically valid, and potentially derive new EQSs according to current regulatory guidance.

ii Objectives

The project detailed within this report had four objectives.

- To perform a literature search of all the available usage, routes to the marine environment, fate and behaviour and ecotoxicity of emamectin benzoate.
- To appraise all of the available data against current regulatory guidance for environmental risk assessment.
- If necessary, derive Predicted No Effects Concentrations (PNECs) for the water and sediment environment.
- Propose appropriate Environmental Quality Standards (EQS) for the protection of organisms in the marine environment.

iii Benefits

Environmental Quality Standards (EQSs) are derived to ensure the adequate protection of marine life that may be potentially exposed to emamectin benzoate. Understanding of the toxicology of emamectin benzoate to various marine taxa has improved since the original risk assessment in 1999, and therefore, it is important to ensure that any EQSs that are proposed are justifiable based on these new data and are suitable to ensure adequate protection of the marine environment.

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iv Conclusions

This project has proposed new EQS values which were derived following current regulatory guidance using a pooled freshwater and marine dataset (see table). It is of note that long-term EQSs were considered appropriate for emamectin benzoate in the environment due to its persistence in sediments. As such, the new EQSs proposed include annual average (AA) EQSs to protect organisms in the environment over a longer period of time, as well as MACs to protect marine life against acute effects. Previously, in the original risk assessment, only MACs were proposed.

Substance	Proposed EQS			
	EQS-MAC marine water	EQS-AA marine water	"Near-field" EQS-MAC for sediment	"Far-field" EQS-AA for sediment
Emamectin benzoate	0.0008 µg/l (0.8 ng/l)	0.000435 µg/l (0.435 ng/l)	0.12 µg/kg dry weight (120 ng/kg dry weight)	0.012 µg/kg dry weight (12 ng/kg dry weight)

AA: Annual Average

MAC: Maximum Acceptable Concentration

1. Introduction

1.1 Background

Emamectin benzoate is the active ingredient in the veterinary medicine Slice® which is used to control sea lice in marine cage fish. In 1999, the Scottish Environment Protection Agency (SEPA) undertook a risk assessment on the use of emamectin benzoate in marine cage fish and derived Predicted No Effects Concentrations (PNECs) for the protection of marine life. These PNECs are the basis of the existing Environmental Quality Standards (EQSs) for emamectin benzoate.

The existing EQSs are comprised of:

- a "near-field" sediment trigger value of 7.63 µg/kg wet weight which is applicable to sediment within 25 m of the marine cages for the protection of sediment re-workers below the marine cages;
- a "far-field" sediment Maximum Acceptable Concentration (MAC) of 0.763 µg/kg wet weight for the protection of all marine life; and
- a MAC for the water column of 0.00022 µg/l for the protection of all marine life.

This review was commissioned by SEPA to establish if any new data have become available, if changes in regulation and guidance require the derivation of the PNECs to be updated or whether the initial assessment in 1999 is still valid.

1.2 Derivation of original Environmental Quality Standards

In 1999, the SEPA undertook a risk assessment on the use of emamectin benzoate and derived PNECs for the protection of marine life. These PNECs were then used to produce the existing EQS for emamectin benzoate.

The existing EQS is comprised of a "near-field" sediment trigger value (7.63 µg/kg wet weight) and a MAC "far-field" sediment standard (0.763 µg/kg wet weight) and a MAC marine standard (0.00022 µg/l). The sediment PNECs used as the basis for the EQS were based on the MATC[1] for the most sensitive sediment species (*Arenicola marina*). A safety factor of 100 was applied to the MATC to derive the "far-field" PNEC (for the protection of all life) and a safety factor of 10 was applied to the MATC to the "near-field" PNEC (to act as a trigger value

[1] Geometric mean between the No Observed Effect Concentration (NOEC) and the lowest observed effect concentration (LOEC).

for prioritising the frequency of monitoring). The standard for the water column took the MATC for the most sensitive water column species (*Americamysis bahia)* and applied an additional safety factor of 100 to derive the PNEC for the water column ($2.2x10^{-2}$ µg/l) (WRc, 2000).

A summary of the acute and chronic laboratory studies reported in the WRc review of the risk assessment undertaken by SEPA in 1999 (WRc, 2000, SEPA 1999) are provided in Appendix A for reference. The most sensitive species for the derivation of the marine and sediment PNECs used in the 1999 evaluation are presented in Table 1.1.

Table 1.1 Most sensitive species selected for the derivation of the 1999 PNECs

EQS environmental compartment	Species	Duration	Endpoint/Concentration
Water column	Water column EQS, Mysid shrimp (*Americamysis bahia*)	96 hours	LC50: 0.04 µg/l NOEC (mortality): 0.018 µg/l MATC (mortality):0.02 µg/l
Sediment	Lugworm (*Arenicola marina*)	10 days	LC50: 111 µg/kg NOEC (mortality): 56 µg/kg MATC (mortality): 76.3 µg/kg

The review by WRc in 2000 expressed some reservations about the sediment studies used in the derivation of the PNECs in 1999. Two studies on sediment dwelling organisms were reported in 1999, which had very similar endpoint concentrations. However, no information on the organic matter, carbon content, or clay content of the sediment was provided in either study, which means that it would be difficult to relate these results to different types of sediment. The concentrations in the *Arenicola marina* study (described in Table 1.1) also decreased significantly over the course of the study. Neither study was considered ultimately of better quality than the other (WRc, 2000). The PNECs derived in 1999 were reconfirmed by SEPA in 2004 (SEPA, 2004).

1.3 Review of emamectin benzoate Environmental Quality Standards

This review has considered:

- any changes to the methodologies for deriving EQSs, in line with the following guidance:
    - Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No. 27 Technical Guidance For Deriving Environmental Quality Standard (EC, 2011a); and

- Regulation and Monitoring of Marine Cage Fish Farming in Scotland – A Procedures Manual (SEPA, 2016b);

- any changes in use patterns for this compound;

- any new data on the physico-chemical properties and environmental fate and behaviour of this compound; and

- any new robust and reliable ecotoxicological studies.

The methodology for this review is comprised of the following tasks, which are described in more detail in the following sections:

- a literature search on emamectin benzoate;

- an assessment of data quality to identify robust and reliable studies;

- a review of the data against the requirements of the regulatory guidance, identification of the "key" data to be used in the derivation of an EQS, identification of data gaps and their potential impact on the EQS; and

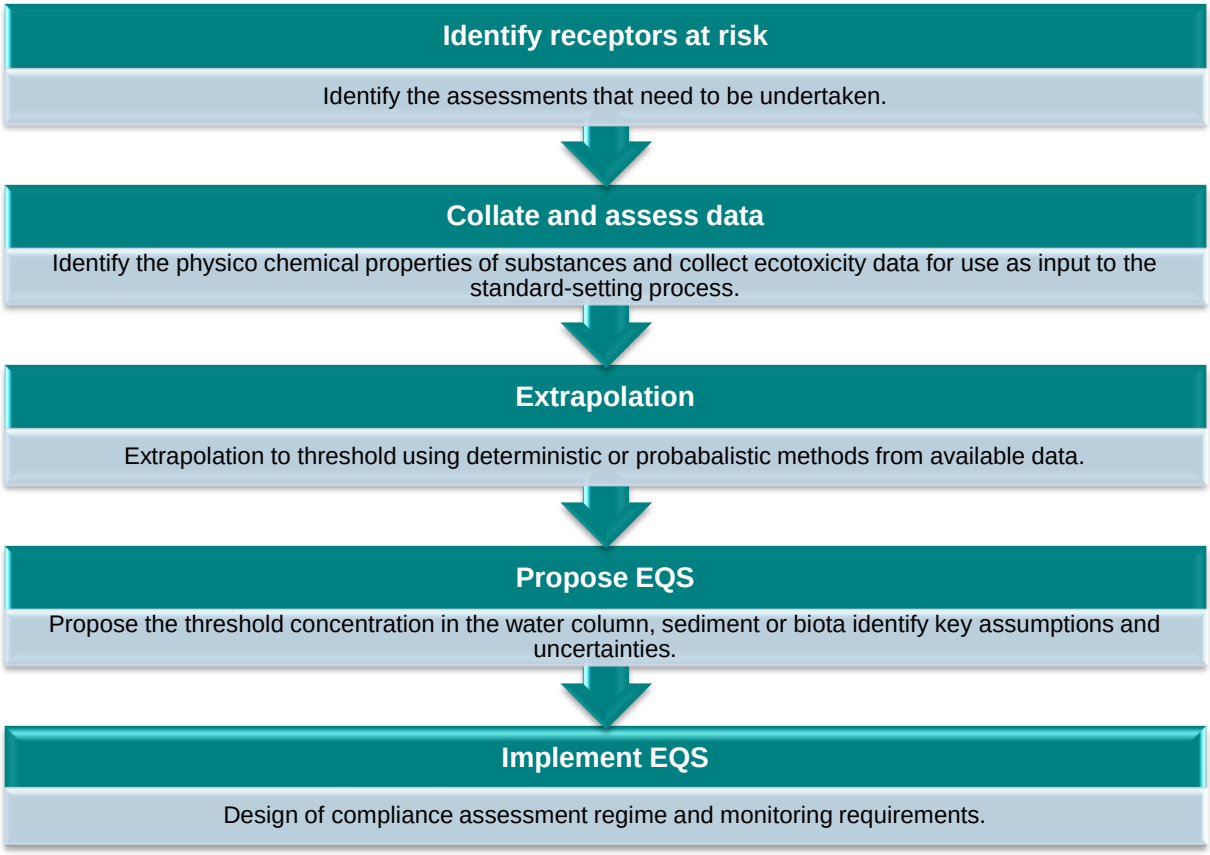
- derivation of an appropriate EQS, outlining details about any uncertainty or assumptions that have been made as part of the derivation.

2. Methodology Adopted

2.1 Scope of Work

The regulatory guidance for deriving PNECs and EQSs is laid out in Guidance Document No. 27 - Technical Guidance for Deriving Environmental Quality Standards (EC, 2011a). This guideline outlines the general steps for deriving an EQS (See Figure 2.1). The approach adopted for this review has followed these general steps, but has been tailored to meet the needs of SEPA especially in regards pragmatic environmental management strategies for use and control of emamectin benzoate release to the environment. It should be noted that whilst this project proposes EQSs for emamectin benzoate, examination of the potential consequences of implementation of the EQS was outside the scope of activities.

Figure 2.1 Overview of steps required to derive an EQS (EC, 2011a)



2.2 Identify receptors and compartments at risk

The receptors and compartments at risk were identified following the collation of the data from various literature sources.

Emamectin benzoate is the active ingredient in the veterinary medicine Slice® which is used to control infestations of sea lice (*Lepeophtheirus sp.* and *Caligus sp.*) in Atlantic salmon (*Salmo salar*) (VMD, 2011). Slice can be used in smolts in freshwater tanks or flowing waterways, and in marine cage fish up to market weight (VMD, 2011). This review covers the use and release of emamectin benzoate to the marine environment (water and sediment) and is therefore, the only compartment considered for environmental release.

The risk assessment in 1999 identified that the marine sediment is the main environmental sink for emamectin benzoate. SEPA derived a "far-field" PNEC standard for marine sediment for the protection of all species, and a trigger value for monitoring as a "near-field" sediment standard. In addition, SEPA also derived a water column short-term PNEC. These PNECs are used as the basis for the existing EQSs.

The data on the environmental fate and behaviour and the strategies for use of emamectin benzoate have been used to inform the most appropriate PNECs for the EQS that will be protective of all species in the marine environment.

2.3 Collate and assess data

2.3.1 Review of 1999 SEPA Risk Assessment

As a baseline, it would have been preferable to examine the risk assessment performed by SEPA in 1999 to understand the data available at the time and the methodology and justifications they used to derive the original PNECs. Unfortunately, it has not been possible to obtain a copy of the original report, and therefore a formal examination of the data was not possible. In 2000, WRc undertook an independent review of the risk assessment that summarised the methodology that was applied by SEPA in 1999, the key data that were available for the evaluation and the justification for the derivation of the PNECs. This review has therefore been used to identify the data that was available at the time, the data gaps and the assumptions that were made to perform the previous assessments and establishes the baseline against which any new information can be appraised.

It should be noted that since the establishment of the Water Framework Directive in 2000 (2000/60/EC) the methodologies, assessment factors and procedures in deriving PNECs have changed from those used in 1999. In Guidance Document No. 27 (EC, 2011a) it is required that data used in the derivation of PNECs is assessed to ensure it is robust and reliable. As it was not possible to identify what methodology was used in 1999 to appraise the quality of this data, it has been assumed that the data underwent a suitable level of quality assurance appropriate for the requirements at the time and are still valid.

2.3.2 Collation of literature data

Data requirements under Guidance Document No. 27 (EC, 2011a) for the derivation of an EQS include:

- data on the physico-chemical properties of the compound - including the fate and behaviour in the environment;

- ecotoxicological data – the data required will be dependent on the environmental compartment that is at risk, and whether the EQS required is long-term or short-term; and

- mammalian toxicity data – this data is only required if it is believed that there may be a risk of secondary poisoning.

To this end a literature search was undertaken to identify all of the currently available data for the relevant data end points. For the purposes of deriving PNECs for emamectin benzoate the relevant data required included:

- data on ecotoxicity of emamectin benzoate for as many different trophic levels as possible mostly preferably but not exclusively in the marine environment;

- data on the fate and behaviour of emamectin benzoate in the environment; and

- data on the use or changes in use, of emamectin benzoate in the last 10 years.

A tiered, robust search strategy was developed, with the most reliable sources of data being explored in the first tier, and less reliable sources searched only if data were limited in the earlier tiers. The order of priority of data sources was as follows:

1. authoritative data sources such as reviews or risk assessments by internationally recognised authoritative bodies (e.g. the World Health Organization, the Organisation for Economic Cooperation and Development and the Food and Agriculture Organization) and regulators within the European Union or globally (e.g. European Commission, European Chemicals Agency, European Food Safety Authority, Defra, Health and Safety Executive, Food Safety Authority, United States Environment Protection Agency and Environment Canada);

2. peer reviewed journals (SCOPUS);

3. data from specific projects investigating the environmental effects of emamectin benzoate;

4. available data in dossiers submitted to regulatory bodies such as REACH dossiers or biocide dossiers;

5. material safety data sheets etc.

The available data sources have been collated into an excel spread sheet table supplied to SEPA as a list of references.

2.3.3 Quality assessment of the data used to generate EQSs/PNECs

The ecotoxicological data provided for different taxonomic groups (e.g. algae, aquatic plants, invertebrates and fish) has been assessed with regard to their reliability and relevance using the Klimisch Criteria. The Klimisch Criteria uses a system of four categories which are described in detail in Table 2.1.

Table 2.1 Summary of the Klimisch Criteria[1]

Code	Category	Description
1	Reliable without restrictions	Refers to studies/data carried out or generated according to internationally accepted testing-guidelines (preferably GLP2) or in which the test parameters documented are based on a specific (national) testing guideline (preferably GLP), or in which all parameters described are closely related/comparable to a guideline method.
2	Reliable with restrictions	Studies or data (mostly not performed according to GLP) in which the test parameters documented do not comply totally with the specific testing guideline, but are sufficient to accept the data or in which investigations are described that cannot be subsumed under a testing guideline, but which are nevertheless well-documented and scientifically acceptable.
3	Not reliable	Studies/data in which there are interferences between the measuring system and the test substance, or in which organisms/test systems were used that are not relevant in relation to exposure, or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for an assessment and which is not convincing for an expert assessment.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

Notes
[1] – Klimisch, H-J., Andreae, M. and Tillmann, U. 1997 *A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.* Regulatory Toxicology and Pharmacology, **25**, 1–5.
[2] - OECD Principles of Good Laboratory Practice (GLP). See: http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Environmental chemistry data and usage data was also scored using a similar system developed by WRc to the Klimisch criteria. The following scores were established for assessing the robustness and *reliability of the chemistry and usage data (See Table 2.2).*

Table 2.2 Scoring criteria for environmental fate and usage data

Code	Category	Description
1	Reliable without restrictions	Data was reported in an authoritative evaluation in Tier 1 or 2 of the literature review and the methodology of the study has been reported and evaluated as acceptable by the authoritative body.
2	Reliable with restrictions	Data was reported in an authoritative evaluation in Tier.1 of the literature review with no further information. Data was reported in literature from Tiers 3 or 4 of the literature review. Study data is available or it has been evaluated as acceptable by the reporting body.
3	Not reliable	Data was reported in literature from Tier 5 of the literature review. No further data is available.
4	Not assignable	Studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature.

As a first screening, only data that were assigned a category 1 or 2 in either scoring system were used in the assessment. However, if no relevant data were available data scored 3 or 4 were used with caution.

2.3.4 Usage and occurrence data

Data on the usage and occurrence of emamectin benzoate were limited to the marine environment in Scotland. The main sources for usage information are from the Summary or Product Characteristics (SPC) obtained from the Veterinary Medicines Database, the SEPA fish farm manual and the aquaculture database provided by SEPA, and the Maximum Residue Level information provided by the European Commission (EC) and the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) (SEPA, 2016a, SEPA, 2016b, VMD, 2016, WHO, 2014, EC, 2016).

The SPC provided information on the method of administration of emamectin benzoate to fish including the dose method and rate, timing and frequency of application recommended for effective control of sea lice. The information provided by SEPA gave data on the licence conditions under which each fish farm are allowed to use emamectin benzoate for protection of the farmed fish and the environment. The MRL information provided by the EC and JECFA gives additional information on the recommended timings of administration to ensure that the MRLs are met when the fish are harvested. Data on the actual reported use of emamectin benzoate in Scottish marine environments were also sought from the Aquaculture website.

All of these data were assessed to establish the rate of release to the environment. The PNEC should account for the use pattern of emamectin benzoate to ensure adequate protection of the marine environment, i.e. emamectin benzoate may be used intermittently (e.g. dosed once over a short period of a week then no further dosing for 12 months) or chronically (e.g. dosed every week for an extended period). The length of potential exposure informed the type of ecotoxicity data that would be relevant for the derivation of the PNEC.

2.3.5 Environmental fate

Data on the environmental fate and behaviour of emamectin benzoate were collated mostly from authoritative evaluations such as the Draft Assessment Report submitted to the European Commission (EC). Data on the solubility, environmental partitioning, biodegradation and biotic and abiotic degradation processes, such as hydrolysis and photolysis were collected. Bioaccumulation and bioconcentration data were also examined. The data were assessed to establish the potential environmental sink, i.e. the environmental compartment that requires protection (sediment and/or water column), and the persistence in the environment or duration of potential exposure. The latter are needed to identify the approach and type of PNEC derived.

2.3.6 Ecotoxicological data

Each ecotoxicological study that was not included in the 1999 assessment was examined and assigned a Klimisch code. This includes data that was available at the time of the first report (i.e. published before 1999) but was listed in the WRc (2000) report. Each study was summarised and assessed for its reliability and robustness. These data were then aggregated with the data used in the 1999 assessment and the key studies for the derivation of appropriate PNECs were selected from all of studies available.

As required by Guidance Document No. 27 (EC, 2011a), the PNEC derivation considered: protection of all receptors from exposure via all routes, analytical capabilities for monitoring, and appraisal against and field or microcosm studies.

In order to select appropriate ecotoxicity data to make this assessment the following points were considered:

- tests at different taxonomic levels are needed to ensure adequate protection of an environmental compartment (e.g. water column or sediment);
- the range of taxa represented in standard tests is ‘modest’, but should protect against substances with different modes of action;
- test species selected are intended to be relevant and representative of particular taxonomic groups;

- test species are generally those suited to use in the laboratory (i.e. can be easily cultured/maintained with low levels of mortality);

- test methods have been standardised by international bodies such as the Organisation for Economic Cooperation and Development (OECD) or the International Standards Organisation (ISO);

- tests will have undergone development and validation phases.

The key ecotoxicological data are selected based on the type of PNEC required. For example, if marine organisms are expected to be chronically exposed to the emamectin benzoate then chronic data are preferential. In addition, if emamectin benzoate is expected to partition to the sediment, then ecotoxicity data for sediment dwelling organisms would be preferred. This is of course limited by the type of studies available, therefore increased uncertainty may have to be considered in the derivation of the PNEC if data gaps exist. The most sensitive receptors were also identified at this stage.

It should be noted that for the derivation of marine PNECs, toxicity data from freshwater species can be "pooled" with toxicity data from marine species to bolster the dataset and reduce uncertainty in the derivation of the PNECs if certain criteria are met. The assumption that freshwater and marine organisms are similarly sensitive to emamectin benzoate must be tested statistically if there are enough data to make statistical analysis workable. This procedure is laid out in by Guidance Document No. 27 (EC, 2011a). If it can be statistically shown that the two sets of species have similar sensitivities then the data can be pooled and used for the derivation of the PNECs. If there are too few data for analysis and no further indication that freshwater and marine organisms have a difference in sensitivity, then the data can be pooled.

2.4 Extrapolation – derivation of threshold concentrations

Once the environmental sink, duration of potential exposure and key ecotoxicity studies had been established, these data were used to propose appropriate PNECs.

2.4.1 Approach adopted

Short- or long-term PNECs for marine waters were derived using the key reliable data following the procedure defined in Guidance Document No. 27 (EC, 2011a). The key factors that are important in the derivation of the EQSs are:

- the nature and extent of the available data set (for both freshwater and marine species);

- the approach adopted, which could be either deterministic (using assessment factors applied to a critical datum) or probabilistic (using Species Sensitivity Distribution modelling); and

- the assessment (safety) factor applied which needs to take account of parameters such as:
    - intra- and inter-laboratory variation of the toxicity data;
    - intra- and inter-species variations (biological variance);
    - short-term to long-term toxicity extrapolation; and
    - laboratory data to field impact extrapolation.

If enough data are available, then a probabilistic approach should be considered, which takes all of the appropriate available data and creates a Species Sensitivity Distribution Model, which provides less uncertainty due to larger size of the data set.

A deterministic approach may be required if the data set is small. It should be recognised that where data are limited there is greater uncertainty associated with the derived PNECs due to the greater magnitude of the assessment (safety) factor that has to be used.

2.5 Propose Environmental Quality Standard

The PNECs that have been derived can be used to inform an appropriate EQS. As part of the EQS proposal, all of the key assumptions and uncertainties are presented in order to provide transparency. It is noted that an EQS must be appropriate for the chemical and the environment, and must fulfil the requirements to allow SEPA to effectively manage the licencing and monitoring or marine cage farms. As such, some work on the occurrence of emamectin benzoate in the Scottish marine environment and data from the monitoring program for the licencing the use of this substance has been presented to provide comparison of the potential impact such an EQS will have.

3. Collate and Assess Data

3.1 Identity of substance

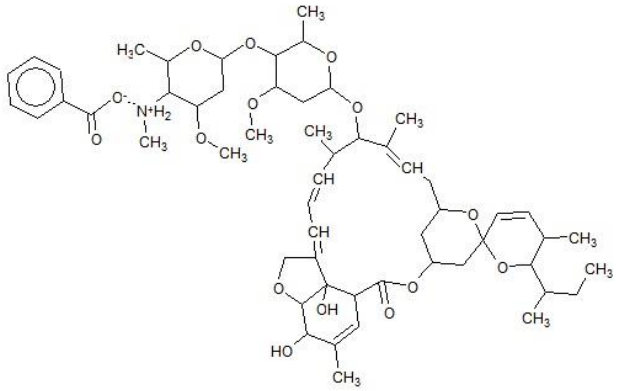
Emamectin benzoate (CAS RN: 155569-91-8 (formerly 137512-74-4 and 179607-18-2)) is semi-synthetic derivative of a chemical produced by the bacterium, *Streptomyces avermitilis*. It is among the class of compounds referred to as avermectins which are used to control internal and external parasites in a wide range of host species, particularly mammals.

Emamectin benzoate is a mixture of two avermectin homologues 4'-epimethyamino-4-deoxyavermectin B_{1a} benzoate (≥90% concentration) and 4'-epimethyamino-4-deoxyavermectin B_{1b} benzoate (≥10% concentration.) (Environment Canada, 2005, WHO, 2014).

3.2 Physical and chemical properties

Table 3.1 summarises the physical and chemical properties of the substance of interest. These end-points have all been given a 1 or 2 classification for reliability.

Table 3.1 Physical and chemical properties of Emamectin benzoate

Property	Value	Method of analysis	Reference
Chemical name	4'-epimethyamino-4-deoxyavermectin B_{1a} benzoate (≥90%) 4'-epimethyamino-4-deoxyavermectin B_{1b} benzoate (≥10%)	-	Environment Canada (2005)
Molecular formula	B_{1a}: $C_{56}H_{81}NO_{15}$ ($C_{49}H_{75}NO_{13}.C_7H_6O_2$) B_{1b}: $C_{55}H_{79}NO_{15}$ ($C_{48}H_{73}NO_{13}.C_7H_6O_2$)	-	EFSA (2012), EC (2011b)
Molecular weight	B_{1a}: 1008.26 g/mol B_{1b}: 994.24 g/mol	-	Environment Canada (2005)
Molecular structure		-	EFSA (2012), EC (2011b)
pH	6.0 at 25°C	CIPAC MT 75.3 – equivalent OECD 122 (published in 2013)	EFSA (2012), EC (2011b)
Vapour pressure	Emamectin benzoate hydrate: 4 x 10^{-6} Pa at 21ºC (97.8% purity, 21.1°C)	OECD 104 - gas saturation method. Used hydrate form as anhydrous form expected to have a lower vapour pressure.	EFSA (2012), EC (2011b)

Property	Value	Method of analysis	Reference
Henry's Law constant	1.3 x 10^{-5} Pa x m^3 x mol^{-1} at pH 5 and 21° 1.3 x 10^{-5} Pa x m^3 x mol^{-1} at pH 7 and 21° 1.3 x 10^{-5} Pa x m^3 x mol^{-1} at pH 9 and 21°	Values calculated use of vapour pressure, molecular weight and water solubility. Deemed acceptable by the Rapporteur Member State.	EFSA (2012), EC (2011b)
Solubility in water	pH 5: 320 mg/l at 25ºC (97.8% purity) pH 7: 24 mg/l at 25ºC (97.8% purity) pH 9: 0.1 mg/l at 25ºC (97.8% purity) Salt water: 5.5 mg/l (maximum)	EEC A6; OECD 105 – flask method.	EFSA (2012), EC (2011b), Environment Canada (2005)
Octanol-water partition coefficient (log Kow)	pH 5.07: 3.0 at 23°C pH 7.00: 5.0 at 23°C pH 9.04: 5.9 at 23°C	EEC A8; OECD 107 – shake flask method – water/solvent system. It was questioned whether the surface active properties of emamectin benzoate would lead to poor repeatability of this method. However, in this case, good repeatability and high recoveries proved that the surface activity of emamectin benzoate did not influence the outcome of these tests.	US EPA (2009); Environment Canada (2005)
Soil Organic Carbon-Water Partitioning Coefficient (Koc)	Sandy loam: 278,983 Sand: 728,918 Clay loam: 25,363 Silt loam: 28,325 Reported average: 265,687 (average)	US EPA 163-1 – leaching and adsorption/desorption studies.	EFSA (2012), EC (2011b) US EPA (2009)
Dissociation constant (pKa	pKb: 9.8 (benzoate) at 20°C pKa: 4.2 (benzoic acid) at 20°C pKa: 7.7 (epi-methyl-NH_2^+) at 20°C	OECD 112 – potentiometric titration.	EFSA, 2012, EC (2011b)

3.3 Environmental fate and behaviour

In water, emamectin benzoate is reported to be stable to hydrolysis at pHs 5.2-8.0 but degrades at pH 9 with a half-life of 19.2 weeks. In sunlight, it degrades with half-lives of 1.4-22.4 days, and is not readily biodegradable. A low Henry's Law Constant indicates that volatilisation from water is not likely to occur (EFSA, 2012, Environment Canada, 2005, USEPA, 2009).

In sediment, degradation is slow. Data from dark sediment studies showed that emamectin benzoate partitioned readily to the sediment, and no metabolites were identified (EFSA, 2012). Field studies performed adjacent to a marine cage showed that only four sediment samples out of 59 taken from up to 10 m away from the cage had detectable levels of emamectin benzoate (Environment Canada, 2005).

It is expected that any emamectin benzoate that enters the environment will be tightly bound to soils or sediment (Environment Canada, 2005, EFSA, 2012, EC, 2011b). It is reported that emamectin benzoate in feed or faeces will be adsorbed to particulates. In a water/sediment study after 1 day 33.6-24.2% of the applied active substance was found in the sediment and after 100 days only 0.3% of the applied active substance was found in the water. After 100 days there was no degradation of the emamectin benzoate in the sediment. The dissipation time for 50% of the applied substance (DT50) for water was reported to be 8.7 days mostly due to partitioning to the sediment. The authors reported that that emamectin benzoate is persistent in whole water/sediment systems with a degradation time for 50% of the applied substance (DegT50) of >120 days (EFSA, 2012).

In laboratory studies using marine sediment and water, 2-3% of the applied active substance was recovered in the water. This result was similar following desorption of emamectin benzoate from sediment, which suggests small amounts of desorption may occur (Environment Canada, 2005). It is reported that over time a soluble form of emamectin benzoate in sediments may form an equilibrium with the interstitial water within the sediment, and then potentially into the water above. This has the potential to reduce the concentration of emamectin benzoate in sediments over time. In field studies, silt traps were placed adjacent marine cages. Only 1% of the total emamectin benzoate in the traps was found in the water phase (Environment Canada, 2005).

Table 3.2 summarises the information obtained from the literature on the environmental fate and partitioning of emamectin benzoate.

Table 3.2 Environmental fate and behaviour of emamectin benzoate

Property	Value	Test method	Reference
Hydrolytic stability (DT50)	Stable at pH 5.2-8.0 DegT50 at pH 9 and 25°C: 19.2 weeks	OECD 111 – and GLP. Samples added to sterile buffers at pH 5, 6, 7, 8 and 9 for 6 weeks and sampled at regular intervals. Hydrolysis rate determined by linear regression.	EFSA, 2012 Environment Canada, 2005
Photostability DegT50 (aqueous, sunlight, state pH)	Natural autumn light Phosphate buffer pH 7: 22.4 days Phosphate buffer with acetone sensitiser: 1.4 days Natural pond water: 6.9 days	US EPA Subdivision N, 161-2. Tests included sterile buffer at pH 7 and natural pond water with a pH of 7.4-8.9. Samples exposed to natural autumn light.	EFSA, 2012
Biodegradation	Not readily biodegradable	OECD 301 F; 92/69/EEC, L383A, C4-D and GLP.	EFSA, 2012
Degradation in water/sediment systems	DT50 water: 8.7 days. DegT50 sediment: could not be calculated as there was no degradation in the sediment. DegT50 whole system: >120 days.	OECD 308 – and GLP Two water sediment systems, silt loam and sand taken from UK freshwater lakes.	EFSA, 2012
Distribution in water/sediment systems (active substance)	Water: 0.3% of the applied dose at day 100. Sediment: 33.6-24.2% of applied dose at day 1.	OECD 308 – and GLP Two water sediment systems, silt loam and sand taken from UK freshwater lakes.	EFSA, 2012

Deg50: Degradation time for 50% of the applied substance. DT50: Dissipation Time for 50% of the applied substance.

3.4 Routes to the environment

Emamectin benzoate is the active ingredient in the veterinary medicine Slice® which is used to control sea lice in marine cage fish. Emamectin benzoate is administered to fish via feed and is expected to be released to the marine environment un-metabolised in the fish faeces or as uneaten food (Environment Canada, 2005, Scottish Executive Central Research Unit, 2002).

Willis *et al.*, (2005) report that emamectin benzoate entering the marine environment is associated with particulate material in the form of fish feed and faeces settling on the sea bed and being incorporated into sediments, but also it leaches from medicated feed. It has been reported that emamectin benzoate will leach into the water column from medicated feed at a rate of 5% of the applied substance after 6 hours following application, and 25% after 7 days following application (Willis *et al.*, 2005). Elimination from fish also occurs over a number of days. Data provided by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food additives (JECFA) and the European Agency for the evaluation of Medicinal Products (EMEA) shows that emamectin benzoate is expected to remain in fish tissues for >90 days (more details in Section 3.6) (WHO, 2014, EMEA, 2003). It is also reported that emamectin benzoate is absorbed via the oral route in salmon, trout and cod but elimination is slow with a terminal half-life of 11 days (WHO, 2014). Therefore, faecal emission from fish is expected to occur for some time following dosing.

3.5 Licensing Conditions

To assess the pattern of release to the environment, it is important to establish the conditions under which emamectin benzoate is administered to marine cage fish. It is noted that these are the conditions under which emamectin benzoate is licenced and authorised for use, but this may not be an accurate picture if uses outside of these frameworks are employed.

3.5.1 Veterinary Medicine Licence

In the UK, emamectin benzoate is licenced by the Veterinary Medicines Directorate (VMD) for use in one medicinal product, Slice, for the control of sea lice (*Lepeophtheirus* sp. and *Caligus* sp.) in Atlantic salmon (*Salmo salar*). It is approved for use in smolts (in freshwater just prior to transfer to saltwater) to market weight fish. It is not intended for use in adult fish or for use as brood stock or in smolts in freshwater cages (due to environmental risks) (VMD, 2011).

Emamectin benzoate is administered via coated cylindrical medicated feed pellets. The pellets are coated in Slice at specific fish feed mills according to the feeding rate. The dosing regimen recommended is a feed rate of 0.5% biomass/day (equivalent to 50 µg/kg biomass/day) for 7 days. If the feeding rate is altered then the concentration of Slice in feed must be adjusted. Table 3.3 shows the quantities of Slice and emamectin benzoate applied to feed and its equivalents administered to fish if the feeding rate is adjusted.

Table 3.3 Quantity of Slice administered per feeding rate (VMD, 2011).

Feeding rate (% biomass of fish)	Concentration of emamectin benzoate in feed (mg/kg feed)	Quantity of Slice per 1,000 kg medicated feed (kg)	Quantity of Slice medicated feed per 1,000 kg fish/day (kg)
0.25	20.0	10.0	2.5
0.5	10.0	5.0	5.0
1.0	5.0	2.5	10.0
2.0	2.5	1.25	20.0
3.0	1.67	0.833	30.0
4.0	1.25	0.625	40.0

The maximum recommended number of marine treatments is 5 within a 2-year growth period, with no more than 3 treatments in a 12 month period. Smolts should only be treated if raised in tanks or flowing waterways and transferred to the marine cages 1-2 days after a seven day treatment has ended.

It is also recommended that fish are not treated more than once in the 60 days prior to the first fish being harvested for human consumption in order for the MRL requirements in food fish to be met (VMD, 2011). The JECFA have derived MRLs for the muscle and skin of salmon and trout of 100 µg/kg (WHO, 2014).

3.5.2 Discharge consents issued by SEPA

SEPA's consenting strategy for emamectin benzoate is based on comparison of monitoring data to the EQSs and computer modelling. EQSs for emamectin benzoate are applied in two ways:

- a consent-limiting concentration of chemical permitted within the seabed sediment ("far-field" EQS); or
- a non consent-limiting concentration of chemical permitted within the seabed sediment which, if exceeded, will trigger a requirement for enhanced monitoring ("near-field" trigger value).

Consents aim to ensure that the "far-field" EQS is not exceeded outside of the Allowable Zone of Effect (AZE) on the sea bed. Exceedances of the "near-field" trigger value within the AZEs will trigger additional monitoring. SEPA provide consent for the amount of Slice that can be used in marine cages which subsequently will not lead to an exceedance of the "far-field"

EQSs in both the water and the sediment (also known as the Total Allowable Quantity (TAQ[2]). The timing and TAQ of emamectin benzoate specified in consents are derived using sediment monitoring data and application of spatial modelling. Repeated treatments following the initial 7 days are only allowed upon authorisation of SEPA (SEPA, 2005, 2014, 2016b). The Maximum Treatment Quantity (MTQ) is the maximum quantity recommended for treatment of the biomass of fish as long as it does not exceed the TAQ (SEPA, 2005, 2014, 2016b).

3.5.3 Usage of emamectin benzoate at Scottish fish farms

The SEPA Aquaculture website reports the monthly usage of emamectin benzoate and the current biomass at each site since 2002. These data were examined to identify if there is any increase in usage of emamectin benzoate since 2002. It should be noted that the 2016 data only covers January to September as data for the last three months of the year are not reported.

Figure 3.1 shows the total biomass of fish farmed in Scotland as reported by the Aquaculture database and the annual average mass of emamectin benzoate applied to each kg of biomass. Each of the site application rates (µg/kg biomass/application) were calculated, then these were averaged per year to give this result. It is clear from this graph that on average the amount of emamectin benzoate per kg of biomass has increased from around 26 µg/kg biomass/year in 2002 to a peak of 67 µg/kg biomass/year in 2015.

There also has been an increase of average application rates from around 1.4 applications/site/year in 2002 to 2.68 applications/site/year in 2016. Figure 3.2 shows the total amount of emamectin benzoate applied to Scottish fish farms and the average number of applications/year/site. The total annual loading of emamectin benzoate to the marine environment in Scotland has also increased. It is of note that between 2002 and 2015 the amount of biomass in Scottish fish farms has doubled whereas the total mass of emamectin benzoate used in Scottish fish farms has increased six fold over the same period.

[2] The Total Allowable Quantity (TAQ) is the maximum level of emamectin benzoate that is allowed to be applied in a single 7-day treatment that will not result in an exceedance of the sediment/water EQS.

Figure 3.1 Total biomass farmed in Scotland/year and the annual average mass of applied emamectin benzoate µg/kg biomass/site

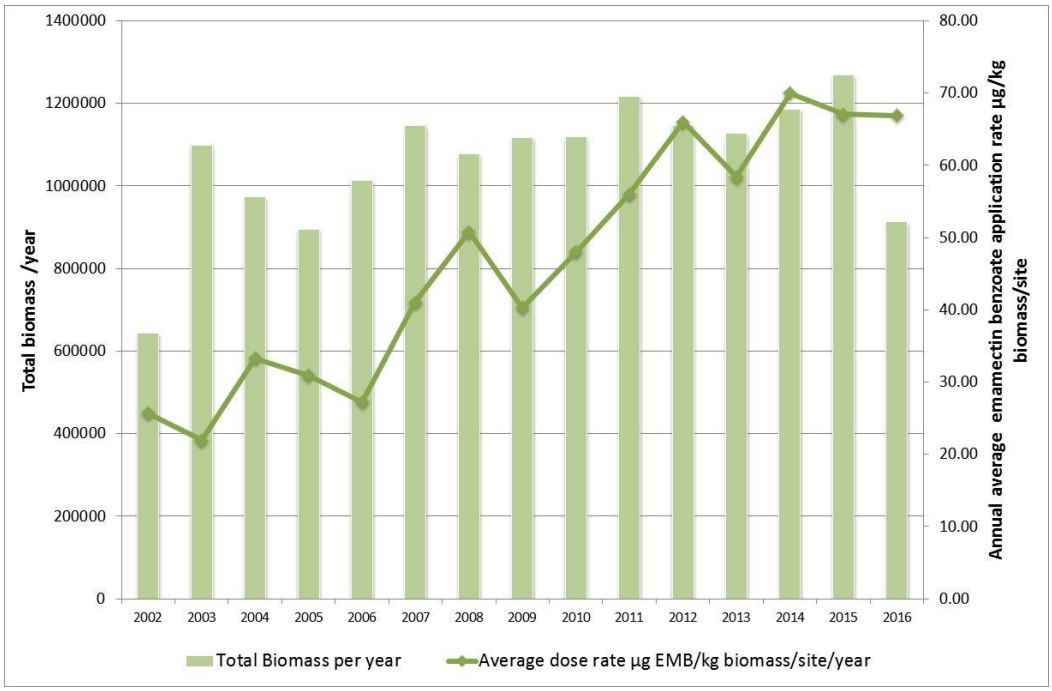
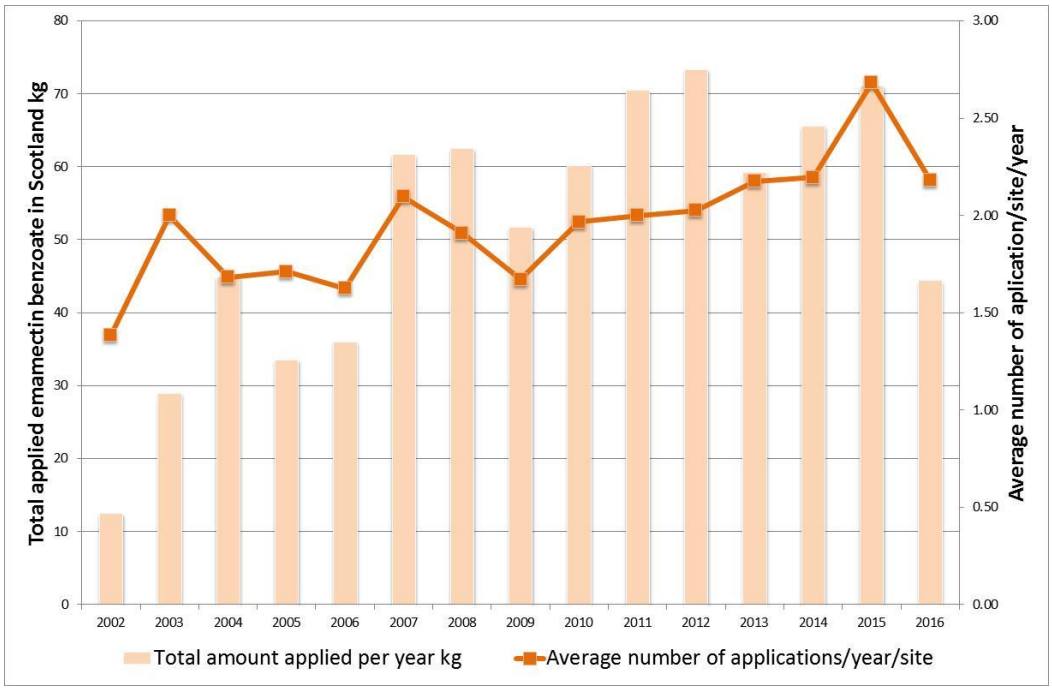


Figure 3.2 Total annual amount of emamectin benzoate applied to Scottish fish farms and the average number of applications/site/year



3.6 Bioaccumulation

One of the beneficial properties of emamectin benzoate as a veterinary drug is its persistence in the skin of fish whereby it can be effective anti-parasitic drug for an extended period of time. In salmon, cod and trout emamectin benzoate is absorbed by the oral route and distributed to the liver, kidneys, skin and muscle. It was reported that the bioavailability of emamectin benzoate in cod is around 38% of the administered dose. In fish, emamectin benzoate is metabolised to various metabolites such as the 8,9-isomer, *N*-demethylated, *N*-formylated, *N*-methylformylated mectins, which are excreted more rapidly than the parent compound. In warmer temperatures, the rate of metabolism in fish is increased (Environment Canada, 2005, WHO 2014).

Atlantic salmon smolts were administered radio-labelled emamectin via gavage at a single dose of 42 µg/kg bw. Peak concentrations in fish tissues were detected at 2-7 days following dosing. The highest concentrations of radio-labelled residues were detected in the bile, liver, and kidney (788 µg/kg at 42 days following administration, 342 µg/kg at 7 days and 361 µg/kg at 21 days, respectively). Lower concentrations were detected in the muscle, skin and brain (13 µg/kg at 4 days, 19 µg/kg at 2 and 28 days and 13 µg/kg at 7 days, respectively). It was not reported if these concentrations were present as un-metabolised emamectin or the relevant metabolites (EMEA, 1999).

Adult Atlantic salmon were also administered emamectin benzoate via feed at a concentration of 50 µg/kg bw/day for 7 days. The fish were then killed at various time points between 3-hours and 45 days following cessation of administration. The concentrations of emamectin benzoate detected in the muscle were 67 µg/kg at 12 hours following cessation of administration and 28 µg/kg at day 30. In skin, 12 hours following cessation of administration, emamectin benzoate was detected at 124 µg/kg and at 39 µg/kg at day 30 (EMEA, 1999).

In a similar study, adult Atlantic salmon were administered 50 µg/kg bw/day emamectin benzoate via feed for 7 days, and killed at various time points between 3-hours and 90 days following cessation of administration. The highest proportion found in the tissues was un-metabolised emamectin benzoate. The concentration of emamectin benzoate in the skin and muscle was 76 µg/kg at 12 hours post-cessation of administration and 19 µg/kg at day 90. It was noted that peak concentrations occurred later than during the study above as the temperature was lower. The proportion of the total radioactive residues in the tissues of the fish that was un-metabolised emamectin benzoate was reduced from 98-100% at 12 hours post-cessation of administration down to 81-89% on day 90. (WHO, 2014, EMEA, 1999).

Ninety days following administration of emamectin benzoate to Atlantic salmon (*Salmo salar*) saw the highest concentrations in the liver and kidneys (Environment Canada, 2005, WHO 2014).

The EFSA reported a Bioconcentration study in Bluegill sunfish (*Lepomis macrochirus*). They reported Bioconcentration Factors (BCF) of 30-102 and 82 l/kg for fillets, viscera and whole

fish, respectively. However, they make note of the fact that these BCFs are for the Total Radioactive Residue (TRR) in the tissues and therefore are very conservative. It is possible that some of these residues are emamectin benzoate metabolites (EFSA, 2012).

Table 3.4 Bioconcentration factors reported for emamectin benzoate

Species	Tissue	Bioconcentration factor (l/kg)	50% Depuration time	Reference
Bluegill sunfish (*Lepomis macrochirus*)	Whole body	80	3.9 days	US EPA (2009) Environment Canada (2005)
Bluegill sunfish (*Lepomis macrochirus*)	Edible Tissue	30	3.8 days	US EPA (2009) Environment Canada (2005)
Bluegill sunfish (*Lepomis macrochirus*)	Non edible tissue	116	4.0 days	US EPA (2009) Environment Canada (2005)
Bluegill sunfish (*Lepomis macrochirus*)	Whole body	69	-	US EPA (2009) Environment Canada (2005)
Bluegill sunfish (*Lepomis macrochirus*)	Flesh	31	-	US EPA (2009) Environment Canada (2005)
Bluegill sunfish (*Lepomis macrochirus*)	Viscera	98	-	US EPA (2009) Environment Canada (2005)

Telfer *et al.* (2006) report on the bioaccumulation of emamectin benzoate in a wide range of indigenous crustaceans, molluscs and fish caught in commercial use conditions at a production fish arm on the west coast of Scotland. Quantifiable levels of emamectin benzoate were detected up to one week post-treatment in the common whelk (*Baccinum undatum*) at 1.08-0.68 µg/kg wet weight and up to one month in the Hermit crab (*Pargurus* spp) at 2.50 µg/kg wet weight. At 12 months post treatment emamectin benzoate was found in a single starfish (*Asterias rubens*) but at a level below the limit of quantification. Mussels (*Mytilus edulis* L.) deployed in bags downstream of fish treatment cages showed levels of emamectin benzoate up to 100 m away at 1 week post treatment but at 10 m by four weeks indicating that it had been accumulated but then largely depurated.

The data suggest that emamectin benzoate remains in the tissues of marine organisms for an extended period (>90 days). This is a property of the substance that is beneficial to its use as a veterinary medicine; as it remains in the fish and the insecticidal properties extend beyond

administration. The metabolism and depuration rate is slow in fish. However, the reported BCFs for whole freshwater fish are marginally lower the threshold for deriving PNECs for secondary poisoning (as prescribed by Guidance Document No. 27 (EC, 2011a)) and therefore these were not deemed necessary for this EQS derivation.

3.7 Mode of action of emamectin benzoate

The mode of action of avermectins generally involves opening glutamate-gated chloride channels at invertebrate inhibitory synapses, which results in increasing chloride concentrations, hyperpolarization of muscle/nerve tissue, and inhibition of neural transmission. Increase in the release of the inhibitory neurotransmitter γ-amino-butyric acid (GABA) is also reported in mammals (Burridge *et al.*, 2008).

3.8 Ecotoxicity data

All of the available ecotoxicity data located are summarised in Appendix A. The assessment of robustness and reliability of each study is listed in the spreadsheet provided to SEPA alongside this document. All concentrations in this report are expressed relative to emamectin benzoate. It is reasonable to assume that published toxicity values are similarly expressed unless otherwise stated; errors will result if this convention has not been observed.

3.8.1 Toxicity to pelagic organisms

Marine organisms

Acute exposure

The acute toxicity data set for marine pelagic organisms includes data for bacteria, crustaceans, molluscs and fish. By far, crustaceans are the taxonomic group to have been studied the most. The most sensitive species in the acute dataset is the mysid shrimp (*Americamysis bahia*) with an LC50 of 0.04 µg/l. This is the same species and endpoint used in the 1999 EQS derivation. WRc did not have access to the original study as this was quoted in the original SEPA risk assessment. It is known that this was deemed an acceptable study in 1999 by SEPA and therefore it has been accepted for the purposes of this review as acceptable.

A 48-hour acute study on a group of copepods collected from Scottish sea lochs reported marginally higher EC50s (0.12-0.57 µg/l) for immobilisation than the mysid shrimp. Even though this study did not report if it used a specific guideline or was performed to GLP, it was well documented and difficulties with ensuring consistent exposure to the test substance during the study were taken into account (such as refreshing the toxicant at regular intervals). This study has therefore been given a Klimisch code 2 “reliable with restrictions” and is acceptable. Larger crustaceans such as lobsters and brown shrimp are reported to be less sensitive to emamectin benzoate than the mysid shrimp and copepods.

The acute studies examined for fish and molluscs reported that they were much less sensitive than the crustaceans, which is expected as emamectin benzoate is intended to control sea lice which are also crustaceans.

Chronic exposure

The available data for chronic ecotoxicity to marine pelagic organisms is limited compared to the acute dataset. Chronic studies were limited to copepods, oysters in early life-stages and long-term studies on mysid shrimp.

The most sensitive species in the chronic dataset was the mysid shrimp (*Americamysis bahia*), with the chronic No Observed Effects Concentration (NOEC) of 0.0087 µg/l. This data point has been repeatedly reported by the US EPA but was provided to the US EPA by a commercial entity and the original study is not available. The US EPA acknowledges that the study had limitations and reported "*Highly erratic test concentrations were observed throughout the study. Measurements were made of dissolved and sorbed material; thus, true dissolved concentrations and toxicity parameters may be lower than reported*". They have, however, used this value in all of the environmental risk assessments for various uses of emamectin benzoate in the USA. As such, despite the limitations, its use by a regulatory authority in their authoritative reports makes this study acceptable for the derivation of a PNEC (USEPA, 2008 and 2009).

Chronic fish and mollusc data show that these species are much less sensitive to emamectin benzoate compared to crustaceans, especially the shrimp.

Freshwater

Acute exposure

The acute dataset for freshwater pelagic organisms contains data for algae, crustaceans, fish and insects. In general, the data report that freshwater fish appear to be slightly more sensitive to emamectin benzoate than marine fish but freshwater crustaceans are less sensitive than marine crustaceans.

The most sensitive freshwater species is reported to be the water flea (*Daphnia magna*) with a 48-hour EC50 of 0.3 µg/l. This is the data point reported in the WRc risk assessment report and is considered robust and reliable enough for the purposes of this review. Additional water flea EC50s that were marginally higher (3.5-11 µg/l) than this value were reported which were conducted according to OECD guidelines 202 and to GLP, as such these were also acceptable for this review.

Chronic exposure

The acute dataset for freshwater pelagic organisms contains data for algae, plants, crustaceans, and fish. Again, crustaceans are the most sensitive freshwater taxa to emamectin benzoate. The lowest chronic freshwater NOEC (0.088 µg/l) is for reproduction in

water fleas (*Daphnia magna).* This was reported in the WRc report (2000) and is therefore considered reliable.

A freshwater microcosm study was located in the EC Draft Assessment Report (EC, 2011). This included phytoplankton, zooplankton and invertebrates. A community NOEC of <0.1 µg/l was derived. The concentrations in the water and sediment were measured but they only reported the nominal concentrations. This study is of limited use to the derivation of the PNEC as the NOEC reported is <0.1 µg/l, which is equivalent to a Lowest Observed Effects Concentration (LOEC), and the true NOEC is unknown. Also as the concentrations reported are nominal concentrations it is possible that this NOEC is actually much lower than reported.

3.8.2 Toxicity to benthic organisms

Marine

Acute exposure

Data for the toxicity of emamectin benzoate to marine benthic organisms is limited. In the acute toxicity dataset only data on annelids and crustaceans were available.

The data for amphipods and lugworm were taken from the WRc report in 2000 and were used in the original risk assessment by SEPA in 1999. The original studies could not be examined in this review but it has been assumed that they are appropriate since they would have been assessed in 1999 by SEPA.

More recently, Veldhoen *et al.* (2012) investigated the biological effects on gene expression within the spot prawn (*Pandalus platyceros*) exposed to emamectin benzoate treated sediment (0.1 – 4.8 mg/kg sediment) in a laboratory seawater aquaria for eight days. Twelve cDNA sequences were isolated from the tail muscle of the crustacean. The study indicates that short term (8 day) exposure can alter mRNA abundance patterns in tail muscle tissue. Three of the transcripts affected by emamectin benzoate (60S ribosomal protein L22, spliceosome RNA helicase WM6/UAP56 and histidine triad nucleotide binding protein 1) suggest that the spot prawn displays a biological response to the chemical, particularly translation/transcription regulation and apoptosis pathways. The mRNA encoding the molting ß-N-acetlyglucosaminidase enzyme was not affected allowing normal growth, development and adult reproduction. No significant differences in weight were observed over the experimental period. However, a LOEC of 42 µg/kg as a measured concentration of emamectin benzoate in wet sediment, was derived based on mortality of prawns. This LOEC is similar to that for sediment dwelling organisms used in the 1999 risk assessment.

Chronic exposure

The only chronic marine species study located in this review was for a 21-day for the polychaete worm (*Capitella capitata*) which reported a NOEC of 460 µg/kg. The robustness of this study could not be assessed as it was not possible to access the original study and it was

only reported briefly in a non-authoritative report. As this study is not reported in any authoritative reports it has been designated a Klimisch code 4 “No assignable”.

Freshwater

Chronic

Only one chronic study was located for benthic freshwater organisms. The literature search located a 28-day *Chironomus riparius* development and emergence study which reported a NOEC for emergence of 1.175 µg/kg. This study has been reported by the EFSA in 2012 and detailed in the Draft Assessment Report for emamectin benzoate in 2011 (EFSA, 2012, EC 2011b). It was conducted according to OECD guideline 218. It was also reported that there was no appreciable decrease in the measured concentrations in the sediment between the start and the end of the study. The difference between the total organic carbon content of the test sediment (4.5%) and the EU standard sediment (5%) is negligible and the result has not been corrected for the carbon content of the sediment. This study is therefore considered appropriate for consideration in the derivation of a PNEC, and as the only chronic sediment study available, is considered a key study in this this process.

3.8.3 Supplementary studies

Additional data for toxicity endpoints that are worthy of note but not relevant for derivation of a PNEC are summarised below.

Marine

Roy *et al.* (2000) reported on tolerance of Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) to emamectin benzoate. Results suggest that Atlantic salmon held at 10 – 14^{0}C at dose rates of at least 173 µg/kg body weight (3.4 x recommended dose) and rainbow trout held at 11 – 13^{0}C at dose rates of at least 218 µg/kg body weight (4.3 x recommended dose) tolerated emamectin benzoate. Signs of toxicity included lethargy, dark colouration and lack of appetite in both species and loss of co-ordination was also reported in Atlantic salmon. Levels of toxicity were identified at dose rates of 356 µg/kg body weight in Atlantic salmon (7.1 x recommended dose) and 413 µg/kg body weight in rainbow trout (8.3 x recommended dose). Fish exposed to the higher nominal dose of 500 µg/kg body weight/day showed no signs of recovery during the 7 day post treatment period. No pathognomonic signs of emamectin benzoate toxicity were obtained during investigation.

Burridge *et al.* (2008) report on several field trials using the optimum therapeutic dose of emamectin benzoate (0.05 mg/kg fish/day) on four cage sites monitoring a total of 1.2 million fish. When treated for seven consecutive days the number of motile and chalimus stages of the crustacean, salmon louse (*Lepeophtheirus salmonis*) was reduced by 94-95% after a 21 day study period. Also, the sea louse, *Caligus elongatus* were present in low numbers suggesting that they were also affected by the treatment. At the end of the treatment

period a significant 80% reduction in motile lice was observed. Another field trial using treated fish reported a similar reduction in sea lice of 68-98%.

Waddy *et al.* (2007) undertook a feeding response study using female American lobsters (*Homarus americanus*) using the commercial medicated salmon feed Slice. Over a two week period the mean ingested dose was below the LOEL for effects on moulting and ranged from 0.02 µg/g to 0.06 µg/g (for organisms at the inter-moult stage and post-moult stage, respectively). The study also showed that lobsters in both the moulting stages preferred natural food rather than medicated pellet food and rejected eating medicated food.

Freshwater

Published data which do not report standard endpoints but provide supplementary freshwater toxicity data are summarised below.

Carcamo *et al.* (2014) undertook a study using emamectin benzoate to determine the effect on mRNA, protein expression levels and enzymatic activity in adult rainbow trout (*Oncorhynchus mykiss*). The study was conducted over a period of 10 – 25 days and followed a seven day medicated feed treatment. Samples of liver, muscle, gill, kidney and intestine tissues showed that the expression and enzymatic activity of cytochrome P450 1A, flavinmonooxygenase and glutathione S-transferase was altered and that this could affect detoxification processes and immunomodulatory mechanisms within the fish species.

Kennedy *et al.* (2014) reported on the effects of avermectins on the inhibition of P-glycoprotein (P-gp) in the blood brain barrier (BBB) of rainbow trout (*Oncorhynchus mykiss*). Clear dose-response relationships were observed using emamectin benzoate over the five doses 0.5, 1.0, 5.0, 10.0 and 50 mg/kg by monitoring neurotoxicity and swimming behaviour of the fish. Swimming ability was affected and reduced when the fish were exposed to cyclosporine A (P-gp substrate) and this suggests that competition for P-gp in the BBB increased emamectin benzoate penetration. The study showed that P-gp protects fish from neurotoxins like emamectin benzoate and ivermectin.

Padilla *et al.* (2012) reported on a developmental *in vitro* test using embryo Zebrafish (*Danio rerio*). The test involved placing embryos individually into Millipore Multiscreen Nylon mesh plates and exposing them to individual chemicals for 5 days post fertilisation. Chemical potencies were then estimated based on numerical calculation using descriptive data (lethality and hatching status). An Activity Concentration for 50% of the population (AC50) of 2.78 µM (equivalent to 2803 µg/l) was reported in this study. This methodology appears to be similar to the Test No. 236: Fish Embryo Acute Toxicity (FET) with some differences.

3.9 Conclusions from literature search

The use of emamectin benzoate suggests that release is continuous for a number of weeks following treatment. Initial releases in a 7-day period will peak as uneaten food is released,

then over a number of weeks the fish slowly excrete un-metabolised emamectin via the faeces. It will significantly partition to the sediment. However, the fate and behaviour data also suggest that, although levels in the seawater are very low, they may form equilibrium with the emamectin benzoate in the sediment. Emamectin benzoate is reasonably persistent in sediments with little degradation through hydrolysis or biodegradation and the use patterns of emamectin benzoate are managed so the current EQS is not exceeded. This suggests that a chronic marine column and sediment PNECs are appropriate for the EQS to protect all receptors at risk.

The most sensitive endpoints from the aggregated data are presented in Table 3.5.

Table 3.5 Most sensitive organisms identified from the aggregated data

Species	End-point/Duration	Concentration µg/l
Mysid shrimp (*Americamysis bahia*)	LC50 (mortality), 96 hours	0.04
Mysid shrimp (*Americamysis bahia*)	NOEC (growth), 28 days	0.0087
Midge larvae (*Chironomus riparius*)	NOEC (emergence), 28 days	1.175

4. Extrapolation – PNEC Derivation

The approach used to derive a particular PNEC is dependent on the amount and type of reliable data available (i.e. whether short- and long-term data is available for a range of species). Based on the extent of the available reliable data an 'assessment or safety factor' is used in the calculation of the PNEC to account for uncertainties surrounding the protection afforded by the proposed value. The aquatic toxicity data must undergo a couple of steps before appropriate PNECs can be derived. The data must be aggregated where more than one data point for each species and endpoint are available. Then analyses are performed to see whether freshwater and marine data can be pooled.

4.1 Derivation of a short-term marine PNEC

A short-term marine PNEC can be derived using the data identified in Section 3 and using the procedure defined in Guidance Document No. 27 (EC, 2011a).

Table 4.1 and Table 4.2 list the aggregated reliable and robust data for the acute marine and freshwater pelagic datasets. The data was collated from the larger dataset (see Appendix A) and species and endpoints were either averaged or the lowest value was used (based on expert judgement on the data available). As recommended in the Guidance Document No. 27 (EC, 2011a), these two datasets were compared using the F-Test, which showed they had unequal variances. A Two-Sample t-Test Assuming Unequal Variances was performed. The result of this is presented in Table 4.3. The results of this test show that the two sets of data are not similar which implies that the two sets of data cannot be pooled.

However, there are concerns with the robustness of this statistical test. It is noted that there are ten data points in the marine dataset. However, the data are heavily weighted towards the crustacean taxonomic group, with seven distinct crustacean data points and only single data points for bacteria, fish and molluscs. In the freshwater dataset there are only seven data points, of which four are for fish species and only single data points for algae, crustaceans and insects. Crustaceans have been shown to be much more sensitive to the effects of emamectin benzoate than fish which means both datasets may be skewed towards the most and least sensitive in each dataset. Seven data points in the freshwater dataset is also quite small and adequate statistical analysis may not be appropriate with this sample size. It therefore does not seem appropriate to rely on the statistical analysis alone to decide if the datasets can be pooled or not.

In general, the datasets appear to be similar with crustaceans as the most sensitive organisms and the fish being much less sensitive. The mode of action for emamectin benzoate is specific to crustaceans and insects and therefore it is expected that they would be the most sensitive. The acute marine pelagic dataset is more extensive than the freshwater but lacks data on algae and only has one fish data point. It is suggested that as the datasets

are not very different (taking into account the potential bias in the datasets), the freshwater data is used to supplement the gaps in the marine dataset.

Table 4.1 Aggregated acute marine pelagic species data

Common name	Scientific name	End point	concentration (µg/l)
Bacteria	*Vibrio fischeri*	EC50 (bioluminescence)	6300
Brown shrimp	*Crangon crangon*	LC50	166
Copepod	*Acartia clausi*	EC50 (immobilisation)	0.57
Copepod	*Pseudocalanus elongatus*	EC50 (immobilisation)	0.12
Copepod	*Temora longicornis*	EC50 (immobilisation)	0.23
Copepod	*Oithona similis*	EC50 (immobilisation)	15.8
Mysid shrimp	*Americamysis bahia*	LC50	0.04
Norway lobster	*Nephrops norvegicus*	LC50	572
Sheepshead minnow	*Cyprinodon variegatus*	LC50	1430
Eastern oyster	*Crassostrea virginica*	EC50 (immobilisation)	490

Table 4.2 Aggregated acute freshwater pelagic species data

Common name	Scientific name	End point	Concentration (µg/l)
Green algae	*Pseudokirchneriella subcapitata*	EC50 (growth inhibition)	9.65
Water flea	*Daphnia magna*	EC50 (immobilisation)	3.5
Rainbow trout	*Oncorhynchus mykiss*	LC50	176
Fathead minnow	*Pimephales promelas*	LC50	384
Carp	*Cyprinus carpio*	LC50	180
Bluegill sunfish	*Lepomis macrochirus*	LC50	194
Asian tiger mosquito	*Aedes albopictus*	LC50	90

Table 4.3 t-Test: Two-Sample Assuming Unequal Variances for the marine and freshwater pelagic acute data

	Marine data	Freshwater data
Mean	1.26	1.84
Variance	3.66	0.59
Observations	10	7.00
Hypothesized Mean Difference	0	
df	13	
t Stat	-0.86	
P(T<=t) one-tail	0.20	
t Critical one-tail	1.77	
P(T<=t) two-tail	0.41	
t Critical two-tail	2.16	

A deterministic approach has been chosen as there is not enough data on enough taxonomic groups to produce a robust Species Sensitivity Distribution curve. The guidance recommends at least eight different taxonomic groups whereas this dataset provides only seven.

There are acute studies available for marine bacteria, crustaceans, fish and molluscs. The marine dataset lacks acute algae data but it is suggested that the freshwater algal data be used to supplement this dataset as discussed above. Using the lowest LC50 of 0.04 µg/l, which was based on results from a 96-hour mortality test on mysid shrimp (*Americamysis bahia*), and applying an Assessment Factor (AF) of 50, a short-term marine PNEC of 0.0008 µg/l (0.8 ng/l) is derived. The AF has been selected on the basis that there are at least three acute freshwater and marine end-points for the key taxonomic groups and acute end-points for an additional marine mollusc. It is also known that the mode of action for emamectin benzoate is specific to crustaceans and insects; therefore as this PNEC is based on the most sensitive crustacean it should be suitable to protect all marine species.

4.2 Derivation of a long-term marine PNEC

A long-term PNEC for marine waters can be derived using the data identified in Section 3 and using the procedure defined in Guidance Document No. 27 (EC, 2011a). There is not enough data available to determine statistically if the two datasets report a similar sensitivity or not (see Appendix A). The only taxonomic group that has been studied in both datasets is crustaceans, which have similar sensitivities in both groups. The mode of action is also specific to crustaceans and insects and therefore it is expected that they will be the most sensitive, therefore the freshwater data has been used to supplement the marine dataset.

A deterministic approach has been chosen as there are not enough reliable data to produce a robust Species Sensitivity Distribution curve.

There are relevant chronic data available for marine crustaceans and molluscs, freshwater algae, daphnia and fish. Using the lowest NOEC of 0.0087 µg/l, which was based on results from a 28-day reproduction test on mysid shrimp (*Americamysis bahia*), and applying an AF of 20, a long-term marine PNEC of 0.000435 µg/l (0.435 ng/l) is derived. The AF has been selected on the basis that there are three chronic freshwater end-points and an additional chronic end-point for a marine mollusc (the mysid shrimp is not considered an additional marine species as it is similar to the freshwater daphnia). If a chronic end-point for an additional marine species such as an echinoderm (which is specific to the marine environment) was available then an AF of 10 would be appropriate.

4.3 Derivation of a long-term marine sediment PNEC

A long-term PNEC for marine sediments can be derived using the data identified in Section 3 and using the procedure defined in Guidance Document No. 27 (EC, 2011a). A deterministic approach has been chosen as there is not enough reliable data to produce a robust Species Sensitivity Distribution curve.

There are relevant chronic data available for a freshwater sediment invertebrate and acute data for three marine organisms that are sediment re-workers such as the lugworm or inhabit the sea bottom such as the amphipod and the spot prawn. The applicable Assessment Factors for deriving a long-term marine sediment PNEC are listed in Guidance Document No. 27 (EC, 2011b).

Using the lowest NOEC of 1.175 µg/kg, which was based on emergence in a 28-day study on midge larvae (*Chironomus riparius*), and applying an AF of 100, a long-term marine sediment PNEC of 0.012 µg/kg (12 ng/kg, rounded) is derived. The AF has been selected on the basis that there is only one long-term chronic freshwater sediment end-point and three acute marine end-points for species with different life-cycles and feeding mechanisms. It should be noted that this PNEC is derived based on dry weight of sediment. The current standard is based on wet weight sediment.

5. Propose Environmental Quality Standard

The PNECs that have been proposed are listed in Table 5.1. These can then be used to propose EQSs.

Table 5.1 Derived PNECs for the protection of marine communities

Derived PNECs		
Short-term marine	**Long-term marine**	**Long-term marine sediment**
0.8 ng/l	0.435 ng/l	12 ng/kg (dry weight)

5.1 Proposed Marine EQS

In 1999, SEPA published a single EQS for the marine environment based on the PNEC. This standard is described a Maximum Allowable Concentration (MAC) in water. It was thought at the time that short-term PNECs were most important, as the use of emamectin benzoate is intermittent. However, it was considered that the residence time for emamectin benzoate is long, therefore, a chronic quality standard would be appropriate and an additional AF was added to the data to derive the EQS.

Following assessment of all the data a chronic EQS would be most appropriate. Especially as emamectin benzoate is expected to persist in the sediment and form equilibrium with the water above. It is therefore recommended that the EQS for the marine water be set at 0.435 ng/l as an annual average and 0.8 ng/l as a MAC. The datasets for each EQS are significant and the taxonomic group that is expected to be the most sensitive have been used in the derivation. It is therefore expected that this EQS will be protective for all species.

The proposed MAC-EQS (short-term) and AA-EQS (long-term) are both higher than the original MAC-EQS derived in 1999 (0.2 ng/l). The proposed EQS is also different in that previously the EQS was for a short-term MAC-EQS only and this proposal is for a MAC-EQS and an AA-EQS. These values should therefore be protective for all pelagic organisms from short-term acute effects and long term chronic effects.

5.2 Proposed sediment EQS

This EQS is comprised of a “near-field” sediment MAC trigger value (7.63 µg/kg wet weight) to protect sediment reworkers below the cages and a “far-field” MAC sediment standard (0.763 µg/kg wet weight) for protection of all marine life. SEPA are keen to keep similar types of standards for managing the licencing of marine cage farms.

For sediment, according to Guidance Document No. 27 (EC, 2011a) only long-term PNECs are considered appropriate for EQSs. A long-term PNEC for sediment of 12 ng/kg (dry weight) has been derived using the available data. It is proposed that this PNEC is used for protection of all organisms in the environment and is equivalent to the "far-field" Annual Average (AA).

A trigger value or "near-field" EQS can be derived using an AF of 10 to the lowest sediment toxicity NOEC of 1.175 µg/kg to protect sediment reworkers below the cages. This would result in a "near-field" sediment MAC EQS of 120 ng/kg (dry weight). It should be noted that the EQSs for sediments are based on a dry weight concentration not a wet weight as per the previous EQS.

Table 5.2 Proposed EQS for the protection of marine communities

Substance	Proposed EQS			
	EQS-MAC marine water	EQS-AA marine water	"Near-field" EQS-MAC for sediment	"Far-field" EQS-AA for sediment
Emamectin benzoate	0.0008 µg/l (0.8 ng/l)	0.000435 µg/l (0.435 ng/l)	0.12 µg/kg dry weight (120 ng/kg dry weight)	0.012 µg/kg dry weight (12 ng/kg dry weight)

AA: Annual Average

MAC: Maximum Acceptable Concentration

6. Occurrence of emamectin benzoate in marine environments around Scotland

The SEPA Aquaculture database contains monitoring data of sediment samples around each licenced fish farm since 2001. SEPA have reported the levels of emamectin benzoate in sediment at 0, 25, 100 or 150 metres from the fish cages. Three replicates were reported for each sample site at the four distances from the fish cages. These data were extracted and tidied, i.e. removal of text such as ND (not detected) and LOD (Limit of Detection) from the numerical values as it is not possible to say what these minimum reporting values are. The geometric mean was calculated for the three replicates at each sample site and the range and the average of the geometric mean are presented in Table 6.1.

Table 6.1 Residue levels of emamectin benzoate in sediment

Distance margin from fish cage (metres)	Range of residue levels (µg/kg)	Average of residue levels (µg/kg)
0	0.01 – 50.19	3.14
25	0.1 – 11.08	2.20
100	0 – 6.3	0.71
150	0.1 – 8.82	1.38

The average concentrations of emamectin benzoate in sediments at 100 m and 150 m from the sea cages are presented in Figure 6.1. It appears that despite the overall usage of emamectin benzoate increasing in Scotland, the concentrations in sediment have generally decreased which may be a result of effective management of authorisations. It could, however, be a product of changes in monitoring that have not been detected in this analysis. These detected concentrations however, are much higher than the AA-EQS proposed in Section 5.

Figure 6.2 shows a histogram of the yearly average detected concentrations of emamectin benzoate at each site in sediment sampled 100m from the sea cages between 2010 and 2016 (no samples 150 m from the cages were reported for this period). This shows that 1.97% of the sites that sampled 100 m from the cages throughout this period had average annual sediment concentrations within the “far-field” AA-EQS of 0.012 µg/kg dry weight sediment.

The average concentrations of emamectin benzoate in sediments within 25 m of the sea cages are shown in Figure 6.3. The concentrations of emamectin benzoate in sediments close to the sea cages do not appear to have increased significantly. Again the concentrations are above the “near-field” trigger value for additional monitoring. It appears that despite the

overall usage of emamectin benzoate increasing in Scotland, the concentrations in sediment have generally decreased which may be a result of effective management of authorisations.

Figure 6.4 shows a histogram of detected concentrations of emamectin benzoate in sediment sampled from directly beneath the sea cages during 201-2016 (no samples from 25 m away from the cages were reported in this period). This histogram shows that 2% of the samples taken were below the new MAC-EQS "trigger value" (0.12 µg/kg dry weight) for extended monitoring.

Figure 6.1 Average yearly concentrations of emamectin benzoate in sediment 100 m and 150 m from the sea cages

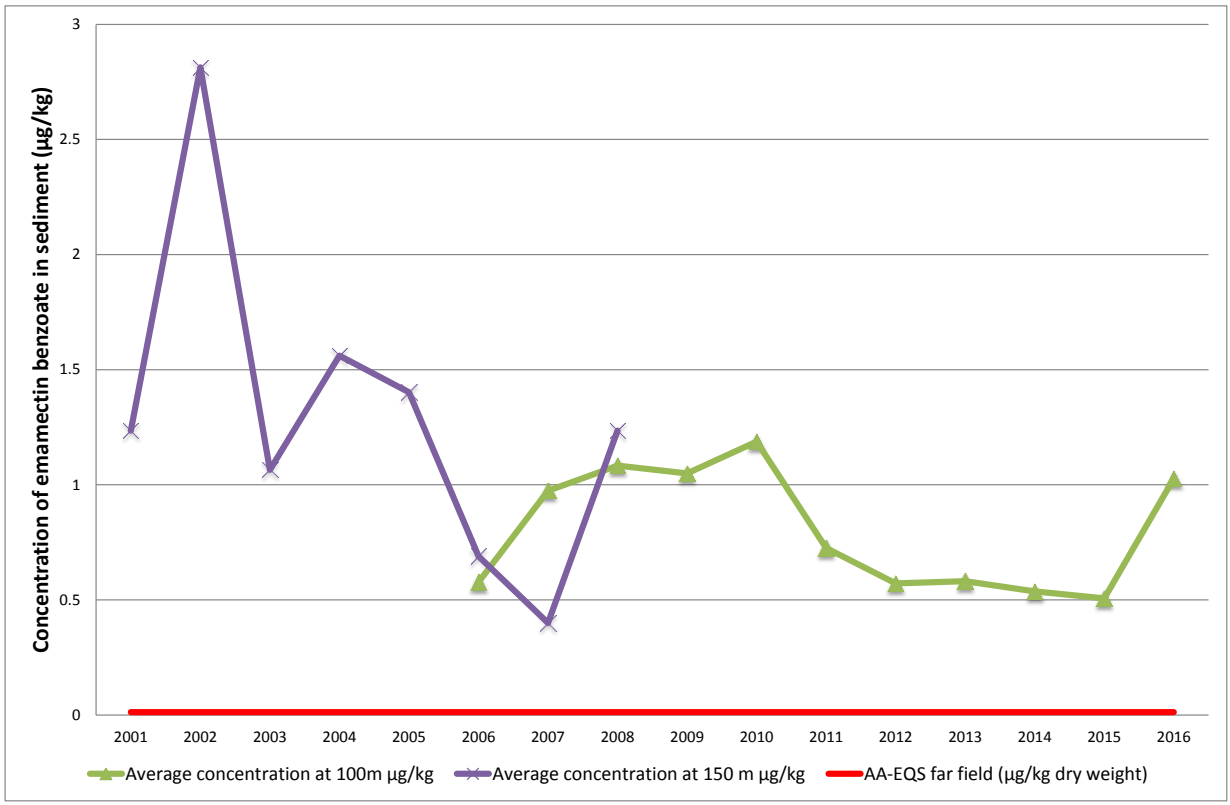
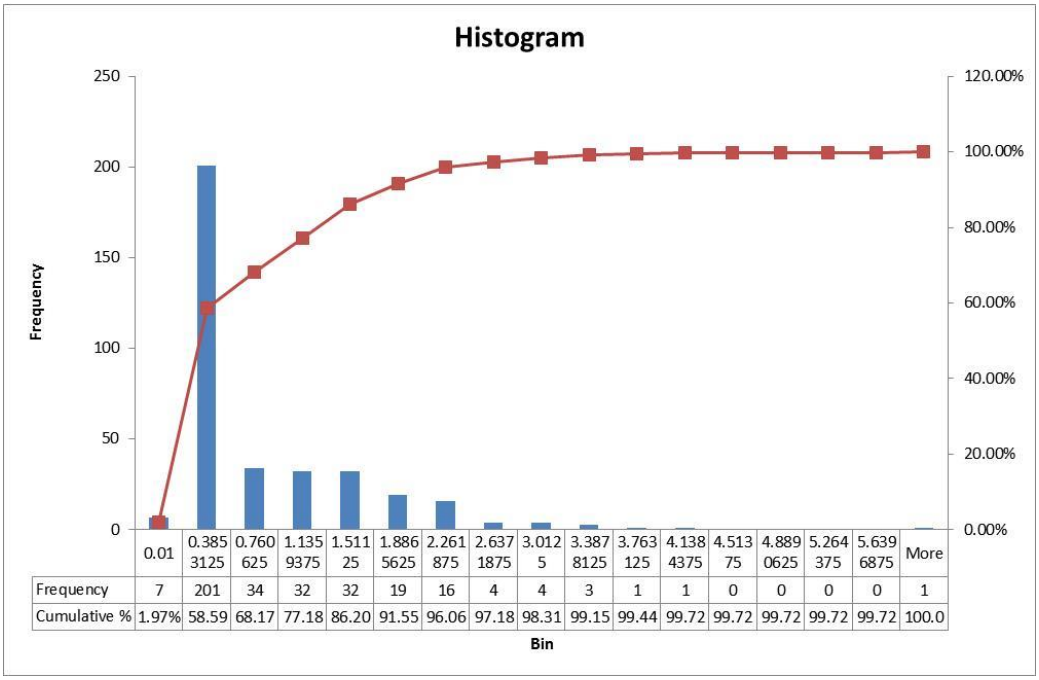


Figure 6.2 Histogram of yearly average concentrations of emamectin benzoate detected in sediment 100 m from the sea cages at each site.



Bin	0.01	0.385 3125	0.760 625	1.135 9375	1.511 25	1.886 5625	2.261 875	2.637 1875	3.012 5	3.387 8125	3.763 125	4.138 4375	4.513 75	4.889 0625	5.264 375	5.639 6875	More
Frequency	7	201	34	32	32	19	16	4	4	3	1	1	0	0	0	0	1
Cumulative %	1.97%	58.59	68.17	77.18	86.20	91.55	96.06	97.18	98.31	99.15	99.44	99.72	99.72	99.72	99.72	99.72	100.0

Figure 6.3 Average yearly concentrations of emamectin benzoate in sediment <25 m from the sea cages

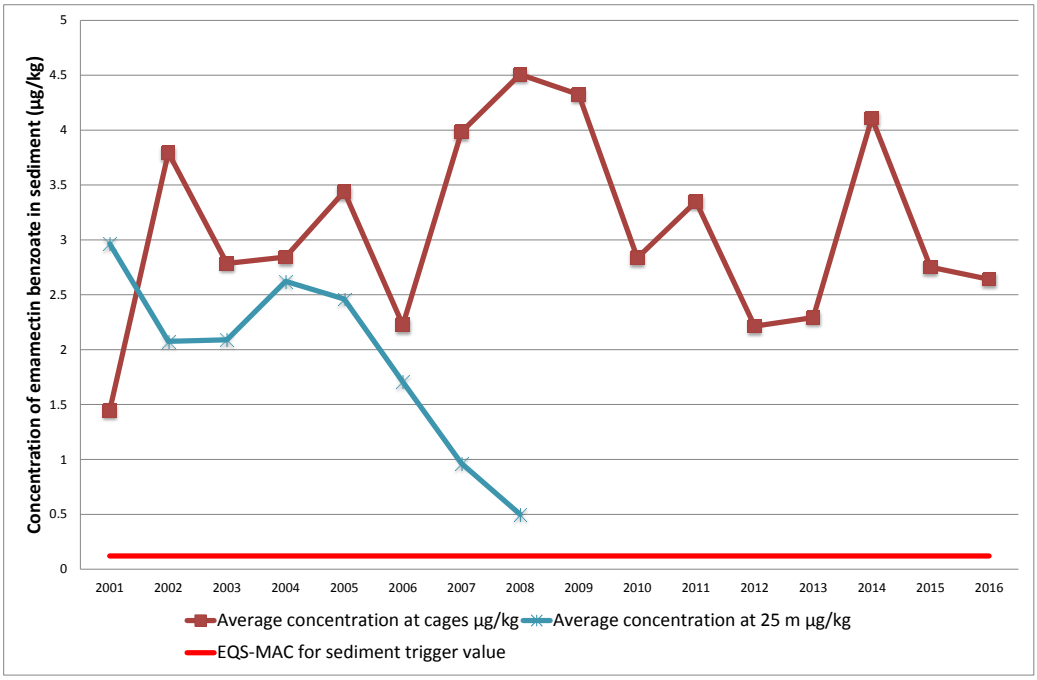
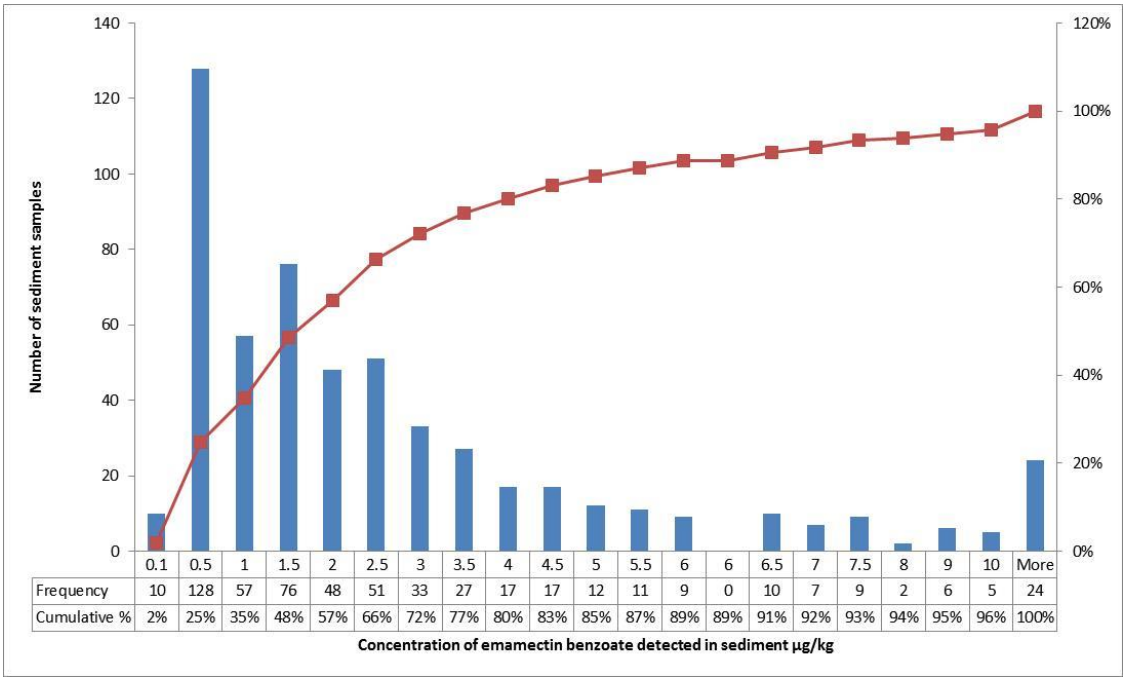


Figure 6.4 Histogram of concentrations of emamectin benzoate detected in sediment directly underneath the sea cages



A literature review was conducted to collect data on the occurrence of emamectin benzoate in Scottish sediment. However, as limited data were located, an additional search was conducted on the occurrence of emamectin benzoate in UK sediment. Data showed that concentrations of emamectin benzoate ranged from 0.25 to 366 µg/kg in the sediment (Table 6.2). These values are all above the proposed MAC-EQS (0.12 µg/kg) and the AA-EQS (0.012 µg/kg dry weight.

Table 6.2 Occurrence of emamectin benzoate in Scottish and UK sediment

Date	Location	Sample details	Concentration	Reference
2010	Scotland	Sediment samples 100 m from fish cages	0.4, 0.6 µg/kg	SEPA, 2012
2010	Scotland	Sediment sample at the fish cage	0.6 µg/kg	SEPA, 2012
Reported in 2002	UK	Sediment	0.25 – 2.73 µg/kg	Boxall *et al.*, 2002
Reported in 2002	UK	Sediment	75.1 – 366 µg/kg	Boxall *et al.*, 2002

7. Conclusions

In 1999, SEPA undertook a risk assessment on the use of emamectin benzoate and derived PNECs for the protection of marine life. These PNECs are the basis of the current EQS for emamectin benzoate. This EQS is comprised of a water quality standard, a “near-field” sediment trigger value and a “far-field” sediment standard.

Currently available data on the use, the routes to the environment, the fate and behaviour and the ecotoxicity of emamectin benzoate have been collated and assessed. Following aggregation of the new data with that used in 1999 and following Guidance Document No. 27 (EC, 2011a), short- and long-term marine PNECs and a long-term marine sediment PNEC have been derived. From these, new EQS values have been proposed (see Table 7.1).

Table 7.1 Proposed EQS for the protection of marine communities

Substance	Proposed EQS			
	EQS-MAC marine water	EQS-AA marine water	“Near-field” EQS-MAC for sediment	“Far-field” EQS-AA for sediment
Emamectin benzoate	0.0008 µg/l (0.8 ng/l)	0.000435 µg/l (0.435 ng/l)	0.12 µg/kg dry weight (120 ng/kg dry weight)	0.012 µg/kg dry weight (12 ng/kg dry weight)

AA: Annual Average

MAC: Maximum Acceptable Concentration

References

Blankinship, A.S., Drottar, K.R., Palmer, S.J., Kendall, T.Z. and Kruegar, H.O. (2002). MK244 SG5 (A10324A): A 48 hour flow through acute toxicity test with the Cladoceran (*Daphnia magna*). Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

Boxall, A.B.A., Fogg, L., Blackwell, P.A. Kay, P. and Pemberton, E.J. (2002) Review of Veterinary Medicines in the Environment. R&D Technical Report P6-012/8/TR.

Burridge, L., Weis, J., Cabello, F. and Pizarro, J. (2008). Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. Available online at: http://www.farmedanddangerous.org/wp-content/uploads/2011/01/SAD_chemicals_report.pdf

Burridge, L.E., Hamilton, N., Waddy, S.L., Haya, K., Mercer, S.M., Greenhalgh, R., Tauber, R., Radecki, S.V., Crouch, L.S., Wislocki, P.G. and Endris, R.G. (2004). Acute toxicity of emamectin benzoate (SLICE^{TM}) in fish feed to American lobster, *Homarus americanus*. Aquaculture Research, 35, 713-722.

Carcamo, J.G., Aguilar, M.N., Barrientos, C.A., Carreno, C.F. and Yanez, A.J. (2014). Emamectin benzoate treatment alters the expression and activity of CYP1A, FMO and GST in different tissues of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 434, 188-200.

EC (2011a) Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No. 27 Technical Guidance for Deriving Environmental Quality Standard

EC (2011b) Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of emamectin in Annex I of the Council Direct 91/414/EEC. European Commission.

EC (2016) European Commission - Pesticides database. Available from: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

EFSA (2009) European Food Safety Authority. Setting of new MRLs for emamectin benzoate in various crops. *EFSA Scientific Report* (2009) 290.

EFSA (2012). European Food Safety Authority. Conclusion on Pesticide risk assessment of the active substance emamectin. EFSA Journal 2012; 10 (11): 2955. Available on request from EFSA: http://dar.efsa.europa.eu/dar-web/provision

EMEA (1999) The European Agency for the Evaluation of Medicinal Products, Committee for Veterinary Products, Emamectin, Summary Report, EMEA/MRL/546/99 Final. January 1999.

Environment Canada (2005). Use of Emamectin Benzoate in the Canadian Finfish Aquaculture Industry – A review of Environmental Fate and Effects. Available online at: http://publications.gc.ca/collections/Collection/En4-51-2005E.pdf

Ezemononye, L.I.N., Ogeleka, D.F. and Okieimen, F.E. (2009). Lethal toxicity of industrial detergent on bottom dwelling sentinels. International Journal of Sediment Research, 24, 479-483.

Helgesen, K.O. and Horsberg, T.E. (2013). Single-dose field bioassay for sensitivity testing in sea lice, Lepeophtherius salmonis: Developmental Screening of the ToxCast Phase I Chemical Library Reprod. Toxicol. 33 (2): 174-187.

Hernando, M.D., De Vettori, S., Martinez Bueno, M.J. and Fernandez-Alba, A.R. (2007). Toxicity evaluation with *Vibrio fischeri* test of organic chemicals used in aquaculture. Chemosphere 68, 724-730.

HSBD (2016). Hazardous Substances Data Bank (HSDB®) database of the US National Library of Medicine. Available online at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB)

Kennedy, C. J., Tierney, K.B. and Mittelstadt, M. (2014). Inhibition of P-glycoprotein in the blood-brain barriers alters avermectin neurotoxicity and swimming performance in rainbow trout. Aquatic toxicology, 146, 176-185.

Khan, H.A.A., Akram, W., Shehzad, K. and Shaalan, E.A. (2011). First report evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. Parasites and Vectors, 4, 146. Available online at: https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-4-146

Klimisch H-J, Andreae M and Tillmann U, 1997 A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology, **25**, 1–5.

Maynard, S.J. (2003a). Emamectin benzoate (MK244): Toxicity to the green algae *Selenastrum capricornutum*. Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

Maynard, S.J. (2003b). Emamectin benzoate:Toxicity to the *Cyprinus carpio*. Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

OECD (2016). OECD eChem Portal. Available online at: http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en

Padilla, S., Corum, D., Padnos, B., Hunter, D.L., Beam, A., Houck, K.A., Sipes, N., Kleinstreuer, N., Knudsen, T., Dix, D.J. and Reif, D.M. (2012). Zebrafish developmental screening of the ToxCast™ Phase I chemical library. Reproductive Toxicology, 33, 174-187.

PPD (2016). Pan Pesticides Database. Available online at: http://www.pesticideinfo.org/ Detail_Chemical.jsp?Rec_Id=PC37430

Roy, W.J., Sutherland, I.H., Rodger, H.D.M. and Varma, K.J. (2000). Tolerance of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum) to Emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture, 184, 19-29.

Schering-Plough Animal Health (2002). Potential environmental impacts of Emamectin benzoate, formulated as SLICE, for salmonids. Schering-Plough Animal Health Technical Report. Animal Pharm. Consulting Group, New Jersey, USA, 33pp. cited by Telfer *et al* (2006).

Science Direct (2016). ScienceDirect® Journal system. Available online at: http://www.sciencedirect.com/

Scottish Executive Central Research Unit (2002) Review and Synthesis of the Environmental Impacts of Aquaculture. The Scottish Association for Marine Science and Napier University Scottish

SEPA (1999). Scottish Environment Protection Agency, Fish Farm Advisory Group. Emamectin Benzoate use in Marine Fish Farms: An Environmental Risk Assessment. SEPA Board Paper 65/99.

SEPA (2004) Regulation and Monitoring of Marine Cage Fish Farming in Scotland – A Procedures Manual Attachment XI Guidance on the use of emamectin benzoate at Marine Cage Fish Farms. Version 1 March 2004

SEPA (2005) Regulation and Monitoring of Marine Cage Fish Farming in Scotland Annex H Methods for Modelling In-feed Anti-parasitics and Benthic effects. Issue date: 18 May 2005

SEPA (2012) Annex 1 – SEPA Habitats Regulations Appraisal of fin fish activity in the Firth of Lorn SAC. SEPA's duties under the Nature Conservation (Scotland) Act 2004 (section 15) and the Conservation Regulations 1994, (Regulations 48 and 49) during regulation. Record of the assessment of the conservation implications of fin fish farm activity, in the Firth of Lorn Special Area of Conservation Licence application number: CAR/L/1099909.

SEPA (2014) Water environment and water services (Scotland) Act 2003 water environment (controlled activities) (Scotland) regulations 2011 ("the regulations"). Water use licence. Licence number: CAR/L/1122569.

SEPA (2016a) Scotland's aquaculture: search the data. Scottish Environment Protection Agency. Available from: http://aquaculture.scotland.gov.uk/data/data.aspx

SEPA (2016b) Regulation and monitoring of marine cage fish farming in Scotland - a procedures manual. Attachment I: Guidance on drafting a Marine Cage Fish Farm Licence.

Telfer *et al* (2006). Environmental effects of the anti-sea lice (Copepoda: Caligidae) therapeutant Emamectin benzoate under commercial use conditions in the marine environment. Aquaculture 260, 163-180.

Tornero, V. and Hanke, G. (2016). Chemical contaminants entering the marine environment from sea-based sources: A review with a focus on European seas. Marine Pollution Bulletin 112, 17-38.

US EPA (2008) Environmental Fate and Ecological Risk Assessment for the Registration of Emamectin Benzoate Use on Tree Nuts and Pistachios (New Use). D345948. July 25,2008.

US EPA (2009). Ecological risk assessment for emamectin benzoate use as a tree injection insecticide to control arthropod pests. PC Code 122806. January 13, 2009. Available online at: https://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-122806_13-Jan-09_a.pdf

US PED (1992). Pesticide Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB)). Environmental Fate and Effects Division, US EPA cited by ECOTOX (2016), EFSA (2012), US EPA (2009) and Environment Canada (2005).

VMD (2011) Veterinary Medicines Directorate. Summary of Product Characteristics Slice 2 mg/g premix for medicated feeding stuff. In Finland only: SLICE Premix for medicated feeding stuff. Revised: October 2011 AN: 01284/2011

Veld, N., Ikonomou, M.G., Bunday, C., Jordan, J., Rehaume, V., Cabecinha, M., Dubetz, C., Chamberlain, J., Pittroff, S., Vallee, K., van Aggelen, G. and Helbing, C.C. (2012). Biological effects of the anti-parasitic chemotherapeutant Emamectin benzoate on a non-target crustacean, the spot prawn (*Pandalus platyceros* Brandt, 1851) under laboratory conditions. Aquatic Toxicology, 108, 94-105.

Volz, E. (2006). Emamectin benzoate metabolite (NOA438306): Acute toxicity to Daphnia magna in a 48 hour immobilisation test. Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

Waddy, S.L., Burridge, L.E., Hamilton, M.N., Mercer, S.M., Aikem, D.E. and Haya, K. (2002). Emamectin benzoate induces molting in American lobster, *Homarus americanus*. Canadian Journal Fish. Aquat. Sci. 59, 1096-1099.

Waddy, S.L., Mercer, S.M., Hamilton-Gibson, M.N., Aiken, D.E. and Burridge, L.E. (2007). Feeding response of female American lobsters, *Homarus americanus*, to SLICE – medicated salmon feed. Aquaculture, 269, 123-129.

Wallace (2001a). Emamectin benzoate: Acute toxicity to Pseudokirchneirella subcapitata. Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

Wallace (2001b). Emamectin benzoate: Acute toxicity to mirror carp (*Cyprinus carpio*) of a 5% SG formulation. Syngenta Crop Protection AG, Basel, Switzerland. Cited by EFSA (2009).

WHO (2014) Evaluation of certain veterinary drug residues in food. WHO Technical Report Series 988. Seventy-eighth report of the Joint FAO/WHO Expert Committee on Food Additives.

Willis, K.J. and Ling, N. (2003). The toxicity of emamectin benzoate, an aquaculture pesticide, to planktonic marine copepods. Aquaculture, 221, 289 – 297.

Willis, K.J., Gillibrand, P.A., Cromey, C.J. and Black, K.D. (2005). Sea lice treatments on salmon farms have no adverse effects on zooplankton communities: a case study. Marine Pollution Bulletin, 50, 806-816.

WRc (2000). Review of SEPA's Environmental Assessment for Emamectin Benzoate. WRc Report Ref: Co 4871/1.

Appendix A Summary of ecotoxicity data available

A1 Marine data

Table A.1 Acute toxicity data for pelagic marine organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Bacteria								
Bacteria	*Vibrio fischeri*	EC50 (bioluminescence)	5, 15, 30 minute	>6300	Static	No toxic effect up to the maximum water solubility. Measured concentration.	2	Hernando *et al* (2007)
Crustaceans								
Brown shrimp	*Crangon crangon*	LC50	192	166	Flow though	Mean measured	2	WRc (2000)
Brown shrimp	*Crangon crangon*	NOEC (mortality)	192	<161	Flow though	Mean measured	2	WRc (2000)
Copepod	*Acartia clausi*	EC50 (immobilisation)	48	0.57 (0.04 – 3.99)	Static	Nauplii life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Acartia clausi*	EC50 (immobilisation)	48	0.28 (0.1 – 0.69)	Static	Copepodite life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Acartia clausi*	EC50 (immobilisation)	48	0.29 (0.08 – 1.1)	Static	Adult life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Pseudocalanus elongatus*	EC50 (immobilisation)	48	0.12 (0.07-0.2)	Static	Nauplii life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Pseudocalanus elongatus*	EC50 (immobilisation)	48	0.14 (0.05-0.44)	Static	Copepodite life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Pseudocalanus elongatus*	EC50 (immobilisation)	48	0.45 (0.22-0.9)	Static	Adult life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Temora longicornis*	EC50 (immobilisation)	48	0.23 (0.12-0.46)	Static	Nauplii life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Temora longicornis*	EC50 (immobilisation)	48	0.41 (0.25-0.67)	Static	Copepodite life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Copepod	*Temora longicornis*	EC50 (immobilisation)	48	2.81 (1.89-4.18)	Static	Adult life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Oithona similis*	EC50 (immobilisation)	48	>15.8	Static	Nauplii life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Oithona similis*	EC50 (immobilisation)	48	15.86 (7.36-34.19)	Static	Copepodite life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Copepod	*Oithona similis*	EC50 (immobilisation)	48	232 (64.5-13586)	Static	Adult life stage. Toxicant analysis not reported.	2	Willis and Ling (2003)
Mysid shrimp	*Americamysis bahia*	LC50	96	0.04	Flow though	Mean measured. Compound stable throughout	2	WRc (2000)
Mysid shrimp	*Americamysis bahia*	NOEC (mortality)	96	0.018	Flow though	Mean measured. Compound stable throughout	2	WRc (2000)
Mysid shrimp	*Americamysis bahia*	MATC (mortality)	96	0.02	Flow though	Mean measured. Compound stable throughout	2	WRc (2000)
Mysid shrimp	*Americamysis bahia*	EC50 (immobilisation)	96	0.04	Flow through	Reported acceptable study. Toxicant analysis not reported.	2	US EPA (2009); EFSA (2009), Environment Canada (2005)
Norway lobster	*Nephrops norvegicus*	LC50	192	572	Flow though	Mean measured	2	WRc (2000)
Norway lobster	*Nephrops norvegicus*	NOEC (mortality)	192	440	Flow though	Mean measured	2	WRc (2000)
Salmon louse	*Lepeophtheirus salmonis*	EC50 (immobilisation)	24	243 (127 – 409)	Static	Salmon and rainbow trout infected with parasites. Parasites collected from a site in an area previously treated with EMB with reported treatment failures. Nominal concentration.	4	Helgesen and Horsberg (2013); ECOTOX (2016)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Salmon louse	*Lepeophtheirus salmonis*	EC50 (immobilisation)	24	51.4	Static	Salmon and rainbow trout infected with parasites. Parasites collected from a site in an area previously treated with EMB with no treatment failures. Nominal concentration.	4	Helgesen and Horsberg (2013); ECOTOX (2016)
Salmon louse	*Lepeophtheirus salmonis*	EC50 (immobilisation)	24	21.5 (18.2 – 23.7)	Static	Salmon and rainbow trout infected with parasites. Parasites collected from a site in an area previously treated with EMB with no treatment failures. Nominal concentration.	4	Helgesen and Horsberg (2013); ECOTOX (2016)
Salmon louse	*Lepeophtheirus salmonis*	EC50 (immobilisation)	24	167 (138 – 199)	Static	Salmon and rainbow trout infected with parasites. Parasites collected from a site in an area previously treated with EMB with reported treatment failures. Nominal concentration.	4	Helgesen and Horsberg (2013); ECOTOX (2016)
Salmon louse	*Lepeophtheirus salmonis*	EC50 (immobilisation)	24	302	Static	Salmon and rainbow trout infected with parasites. Parasites collected from a site in an area previously treated with EMB with reported treatment failures. Nominal concentration.	4	Helgesen and Horsberg (2013); ECOTOX (2016)
American lobster	*Homarus americanus*	LC50	7 days	644 $\mu g.g^{-1}$ food	feeding	Adults. Toxicant analysis not reported.	2	Burridge *et al*. (2004)
American lobster	*Homarus americanus*	LC50	7 days	>589 $\mu g.g^{-1}$ food	feeding	Stage V and VI juveniles. Toxicant analysis not reported.	2	Burridge *et al*. (2004)
American lobster	*Homarus americanus*	EC44 (premature moulting)	≤ 100 days	1 µg/g food	feeding	Adult females. Toxicant analysis not reported.	2	Waddy *et al*. (2002)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Molluscs								
Eastern oyster	*Crassostrea virginica*	EC50 (immobilisation)	96	490 (410 – 590)	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Eastern oyster	*Crassostrea virginica*	LC50	96	670	Not reported	Toxicant analysis not reported.	2	Environment Canada (2005)
Eastern oyster	*Crassostrea virginica*	NOEC (mortality)	96	260	Not reported	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Fish								
Sheepshead minnow	*Cyprinodon variegatus*	LC50	96	1430	Flow though	Mean measured. Discolouration observed at 500 µg/l	2	WRc (2000)
Sheepshead minnow	*Cyprinodon variegatus*	No mortality concentration	96	860	Flow though	Mean measured. Discolouration observed at 500 µg/l	2	WRc (2000)
Sheepshead minnow	*Cyprinodon variegatus*	LC50	96	1430 (1250–1670)	Flow through	Measured concentration.	1	Environment Canada (2005), EFSA (2009) cited 1995 data, EC (2011b)
Sheepshead minnow	*Cyprinodon variegatus*	NOEC (mortality)	96	860	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005)

EC50 = concentration effective against 50% of the organisms tested
LC50 = concentration lethal to 50% of the organisms tested
LOEC: Lowest observed effect concentration
MATC: Maximum Acceptable Toxicant Level.
NOAEC: No Observed Adverse Effect Concentration
NOEC: No observable effect concentration

Table A.2 Chronic toxicity data for pelagic marine organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Crustaceans								
Copepod	*Acartia clausi*	NOEC (fecundity	8 days	0.05	Static	Adult life stage. Nominal concentration but losses minimal as toxicant renewed every 24 hours.	2	Willis and Ling (2003)
Mysid shrimp	*Americamysis bahia*	NOEC (effect not reported)	28 days	0.018	Flow through	Reported supplemental study. Toxicant analysis not reported.	2	US EPA (2009), ECOTOX (2016)
Mysid shrimp	*Americamysis bahia*	NOEC (growth)	28 days	0.0087	Flow through	Reported supplemental study. Toxicant analysis not reported.	2	US EPA (2009), ECOTOX (2016)
Mysid shrimp	*Americamysis bahia*	LOEC (growth, survival and reproduction)	28 days	0.02	Flow through	Toxicant analysis not reported.	4	ECOTOX (2016)
Molluscs								
Eastern oyster embryo	*Crassostrea virginica*	EC50 (shell deposition)	96	530	Flow through	Mean measured. Compound stable throughout	2	WRc (2000)
Eastern oyster embryo	*Crassostrea virginica*	NOEC (shell deposition)	96	260	Flow though*	Mean measured. Compound stable throughout	2	WRc (2000)
Eastern oyster	*Crassostrea virginica*	EC50 (shell deposition or embryo larvae)	Not reported	490	Flow through	Reported acceptable study – Highly toxic. Toxicant analysis not reported.	2	US EPA (2009)
Eastern oyster	*Crassostrea virginica*	EC50 (shell deposition or embryo larvae)	Not reported	530	Flow through	Toxicant analysis not reported.	1	EFSA (2009)

EC50 = concentration effective against 50% of the organisms tested

LOEC: Lowest observed effect concentration

NOEC: No observable effect concentration

Table A.3 Acute toxicity data for benthic marine organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/kg)	Exposure	Comment	Reliability Index	Reference
Annelids								
Lugworm	*Arenicola marina*	LC50	10 days	111	Not reported	Mean measured concentration.	2	WRc, 2000
Lugworm	*Arenicola marina*	NOEC (mortality)	10 days	56	Not reported	Mean measured concentration.	2	WRc, 2000
Lugworm	*Arenicola marina*	MATC (mortality)	10 days	76.3	Not reported	Mean measured concentration.	2	WRc, 2000
Crustaceans								
Amphipod	*Corophium volutator*	LC50	10 days	193	Not reported	Mean measured concentration.	2	WRc, 2000
Amphipod	*Corophium volutator*	NOEC (mortality)	10 days	115	Not reported	Mean measured concentration.	2	WRc, 2000
Amphipod	*Corophium volutator*	MATC (mortality)	10 days	190	Not reported	Mean measured concentration.	2	WRc, 2000
Amphipod	*Corophium volutator*	LC50	10 day	6.32	Not reported	Carried out in the absence of sediment. Mean measured concentration, high control mortality.	2	WRc, 2000
Amphipod	*Corophium volutator*	NOEC (mortality)	10 days	3.2	Not reported	Carried out in the absence of sediment. Mean measured concentration, high control mortality.*	2	WRc, 2000
Spot prawn	*Pandalus platyceros*	LOEC (mortality, genetic changes)	8 days	42	Flow through	Measured concentration.	2	Veldhoen *et al* (2012)
Spot prawn	*Pandalus platyceros*	EC20 (mortality, genetic changes)	8 days	138	Flow through	Measured concentration.	2	Veldhoen *et al* (2012)

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

LOEC: Lowest observed effect concentration

MATC: Maximum Acceptable Toxicant Level

NOEC: No observable effect concentration.

Table A.4 Chronic toxicity data for benthic marine organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/kg)	Exposure	Comment	Reliability Index	Reference
Annelids								
Polychaete worm	*Capitella capitata*	NOEC (effect not reported)	21 days	460	Not reported	No study details available, not used by any authoritative bodies in their assessments.	4	Schering-Plough Animal Health (2000) cited by Telfer et al (2006)

NOEC: No observable effect concentration.

A2 Freshwater data

Table A.5 Acute toxicity data for pelagic freshwater organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Algae								
Algae	*Pseudokirchneriella subcapitata*	EC50 (growth inhibition)	96	12.1	Static	OECD 201, GLP. Measured concentration.	1	EFSA (2009) cited Maynard (2003a)
Algae	*Pseudokirchneriella subcapitata*	EC50 (growth)	96	7.2	Static	Mean measured concentration.	2	EFSA (2012), EC (2011b)
Algae	*Pseudokirchneriella subcapitata*	EC50 (growth inhibition)	96	8170	Static	OECD 201, GLP. Nominal concentration.	3	EFSA (2009) cited Wallace (2001, a)
Crustaceans								
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	1	Flow though	Mean measured concentrations.	2	WRc (2000)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Water flea	*Daphnia magna*	NOEC (mortality and immobilisation)	48	0.3	Flow though*	Mean measured concentrations.*	2	WRc (2000)
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	3.5	Flow through	OECD 202, GLP. Mean measured concentration.	1	EFSA (2009) cited Blankinship *et al* (2002). EC (2011b)
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	11	Static	OECD 202, GLP. Measured concentration.	1	EFSA (2009) cited Volz (2006)
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	1	Flow through	Mean measured concentration.	1	EFSA (2012); EC (2011b), US EPA (2009) refers to 1993 data
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	>728	Static	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Water flea	*Daphnia magna*	EC50 (immobilisation)	48	1 (0.84 – 1.2)	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Fish								
Rainbow trout	*Oncorhynchus mykiss*	LC50	96	174	Flow though	Mean measured concentrations.	2	WRc (2000)
Rainbow trout	*Oncorhynchus mykiss*	NOEC (mortality)	Not reported	49	Flow though	Mean measured concentrations.	2	WRc (2000)
Rainbow trout	*Oncorhynchus mykiss*	LC50	96	180	Flow though	Mean measured concentrations.	2	WRc (2000)
Rainbow trout	*Oncorhynchus mykiss*	NOEC (mortality)	Not reported	87	Flow though	Mean measured concentrations.	2	WRc (2000)
Rainbow trout	*Oncorhynchus mykiss*	LC50	96	174	Flow through	Mean measured concentration.	1	EFSA (2012); EC (2011b), US EPA (2009)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Rainbow trout	*Oncorhynchus mykiss*	NOEC (mortality)	96	49	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Fathead minnow	*Pimephales promelas*	LC50	96	194	Flow though	Mean measured concentrations.	2	WRc (2000)
Fathead minnow	*Pimephales promelas*	NOEC (mortality)	Not reported	89	Flow though	Mean measured concentrations.	2	WRc (2000)
Fathead minnow	*Pimephales promelas*	LC50	96	194	Flow through	Measured concentration.	1	Environment Canada (2005), EFSA (2009), EC (2011b)
Fathead minnow	*Pimephales promelas*	NOEC (mortality)	96	160	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005)
Carp	*Cyprinus carpio*	LC50	96	200	Static	OECD 203, GLP. Measured concentration.	3	EFSA (2009) cited Maynard (2003b)
Carp	*Cyprinus carpio*	LC50	96	567	Static	OECD 203, GLP. Measured concentration.	1	EFSA (2009) cited Wallace (2001b)
Bluegill sunfish	*Lepomis macrochirus*	LC50	96	180 (40 – 240)	Flow through	Nominal concentration.	1	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992), EFSA (2008)
Bluegill sunfish	*Lepomis macrochirus*	NOEC (mortality)	96	90	Flow through	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Insects								
Asian Tiger mosquito	*Aedes albopictus*	LC50	24	90 (40 – 140)	Static	Laboratory study. Nominal concentration.	2	Khan *et al* (2011)
Asian Tiger mosquito	*Aedes albopictus*	LC50	24	1390 - 2450	Static	Lahore field population (Pakistan) . Nominal concentration.	2	Khan *et al* (2011)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Asian Tiger mosquito	*Aedes albopictus*	LC50	24	1350 - 2000	Static	Faisalabad field population (Pakistan). Nominal concentration.	2	Khan *et al* (2011)
Asian Tiger mosquito	*Aedes albopictus*	LC50	24	1140 - 1700	Static	Sargodha field population (Pakistan). Nominal concentration.	2	Khan *et al* (2011)

EC50 = concentration effective against 50% of the organisms tested

LC50 = concentration lethal to 50% of the organisms tested

NOEC: No observable effect concentration.

Table A.6 Chronic toxicity data for pelagic freshwater organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Algae								
Algae	*Pseudokirchneriella subcapitata*	EC50 (abundance and growth)	5 days	>3.9	Static	Reported acceptable study, GLP. Measured concentration.	1	EFSA (2012), US EPA (2009); ECOTOX (2016) cited US Pesticide Ecotoxicity Database (1992)
Algae	*Pseudokirchneriella subcapitata*	NOEC (abundance)	5 days	<3.9	Static	Reported acceptable study, GLP. Toxicant analysis not reported.	1	US EPA (2009) ECOTOX (2016) cited US Pesticide Ecotoxicity Database 1992
Duckweed								
Duckweed	*Lemna gibba*	EC50 (abundance)	14 days	>94	Static	Mean measured concentration.	2	EFSA (2012); US EPA (2009); ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992).
Duckweed	*Lemna gibba*	NOEC (abundance)	Not reported	94	Static	Reported acceptable study. Toxicant analysis not reported.	2	US EPA (2009); ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Crustaceans								
Water flea	*Daphnia magna*	NOEC (mortality)	21 days	88	Flow though	Mean measured concentrations.	2	WRc, 2000
Water flea	*Daphnia magna*	LOEC (mortality)	21 days	160	Flow though*	Mean measured concentrations.*	2	WRc, 2000
Water flea	*Daphnia magna*	NOEC (reproduction)	21 days	0.088	Static	Mean measured.	1	Environment Canada (2005) EC (2011b)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Water flea	*Daphnia magna*	LOEC (reproduction)	21 days	0.16	Static	Toxicant analysis not reported.	2	Environment Canada (2005) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)
Water flea	*Daphnia magna*	NOEC (reproduction)	21 days	0.088	Static	Mean measured.	1	Environment Canada (2005) EFSA, 2009
Water flea	*Daphnia magna*	NOAEC (effect not reported)	'Chronic study' – no further details	0.088	Flow through	Reported acceptable study. Toxicant analysis not reported.	2	US EPA (2009)
Fish								
Fathead minnow	*Pimephales promelas*	NOEC (hatching success, survival and growth)	32 days	12	Not reported	4 day embryo hatch period and 28 day post hatch juvenile growth period. Mean measured concentrations.	2	WRc, 2000
Fathead minnow	*Pimephales promelas*	LOEC (hatching success, survival and growth)	32 days	28	Not reported*	4 day embryo hatch period and 28 day post hatch juvenile growth period. Mean measured concentrations.*	2	WRc, 2000
Fathead minnow	*Pimephales promelas*	MATC (hatching success, survival and growth)	32 days	18	Not reported*	4 day embryo hatch period and 28 day post hatch juvenile growth period. Mean measured concentrations.*	2	WRc, 2000
Fathead minnow (early life stage)	*Pimephales promelas*	NOEC/LOEC (growth)	32 days	12	Flow through	Mean measured concentration.	1	EFSA (2012), EC (2011b), ECOTOX (2016)
Fathead minnow (early life stage)	*Pimephales promelas*	NOEC (effect not reported)	32 days	6.5	Not reported	Toxicant analysis not reported.	2	US EPA (2009) cited ECOTOX (2016) who cited US Pesticide Ecotoxicity Database (1992)

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/l)	Exposure	Comment	Reliability Index	Reference
Microcosm								
Outdoor microcosm	phytoplankton, zooplankton and invertebrates	NOEC (community)	139 days	0.1	Static	Measured concentrations	1	EC (2011b)

EC50 = concentration effective against 50% of the organisms tested

LOEC: Lowest observed effect concentration

MATC: Maximum Acceptable Toxicant Level.

NOEC: No observable effect concentration.

Table A.7 Acute toxicity data for benthic freshwater organisms exposed to emamectin benzoate

Common name	Scientific name	End point	Test duration (hrs)	Conc. (µg/kg)	Exposure	Comment	Reliability Index	Reference
Insects								
Midge	*Chironomus riparius*	NOEC (emergence)	28 days	1.25	Static	OECD 218 guideline. Nominal concentration (measured concentrations were between 94 and 116% of the nominal, therefore 1.25 µg/kg nominal is equivalent to 1.175 to 1.45 µg/kg measured)	2	EC (2011b). EFSA (2012)
Midge	*Chironomus riparius*	NOEC (development)	28 days	10	Static	OECD 218 guideline. Nominal concentration (measured concentrations were between 94 and 116% of the nominal, 10 µg/kg nominal is equivalent to 9.4 to 11.6 µg/kg)	2	EC (2011b)

NOEC: No observable effect concentration.