May 2019

Chemistry Task Team Recommendation for an EQS for Emamectin Benzoate

Executive Summary

UKTAG's Chemistry Task Team (CTT) was asked to form an opinion on environmental quality standards (EQS) for emamectin benzoate, which is used as an active ingredient in fish farm sealice medicines. This opinion is summarised and described in this report.

The Scottish Environment Protection Agency (SEPA) previously set standards for the substance in 1999, before current EQS development guidance and significant new data were available. In forming its opinion, CTT followed the Technical Guidance for Deriving Environmental Quality Standards (2011 WFD Common Implementation Strategy technical guidance 27; herein CIS 27), reviewing data for reliability and relevance where appropriate. Based on the substance's properties and its use pattern, CTT has derived EQS for the marine environment only.

CTT reviewed a number of pieces of information in reaching this opinion:

i. an EQS report commissioned by SEPA and produced by the environmental consultancy WRc in January 2017;
ii. the comments of three independent peer reviewers on the WRc report;
iii. a 2018 SEPA field study conducted in the Shetland isles (including one of the peer reviewer's comments on this);
iv. results of more recent ecotoxicity testing conducted by Industry, and
v. a recent industry-funded fish farm field survey

CTT also considered a recent industry-sponsored EQS derivation report that includes these new data (points iv and v above) that was submitted as part of the data package (wca 2018). CTT have provided a response to this derivation in a separate background document.

The 2017 WRc EQS report identified four EQS: two sediment quality standards (QS), one protective of "near field" effects and the other protective of "far field" effects, as well as two QS for water (protective of marine pelagic organisms) in relation to long term and short term exposures. EQS development for secondary poisoning was not necessary following CIS 27 as the substance has a low potential for bioaccumulation.

As well as the reasonably large quantity of data relating to toxicity in sediment-dwelling organisms that is available for emamectin benzoate, other factors needed to be considered in deriving an EQS for the sediment compartment. Decisions on each of these factors may contribute to either a more or a less stringent approach (ie lower or higher EQS), as described in the body of this report. The factors include: the relevance of the most sensitive tested organism, a freshwater insect, for the marine environment; the differences between the tested marine organisms in terms of their living and feeding strategies; the lack of a true chronic study in the most sensitive marine sediment species in acute tests; and difficulties in

reconciling the conclusions of the two field studies. Given the complexity of this case in relation to the sediment data, CTT explored all possible approaches to derive possible QS allowed for under CIS 27, and used a weight of evidence approach using several lines of evidence to help corroborate the recommended sediment EQS.

The table below summarises the EQS CTT are proposing, along with justifications. The 1999 SEPA standards are included for comparison. Further detail on all of the derivations can be found in the body of this report.

	EQS			
	Sediment EQS (ng/kg dwt)	Sediment "near field" EQS (ng/kg dwt)	Maximum Acceptable Concentration EQS_{water} - Pelagic acute effects (ng/l)	Annual Average EQS_{water} - Pelagic chronic effects (ng/l)
SEPA 1999 standards	760 (rounded)*	7630*	n/a	0.2
CTT recommendation	23.5	n/a	7.8	0.19
CTT justification	Lowest relevant & reliable study (28-day *Chironomus riparius* NOEC 1.175ug/kg); assessment factor 50. -fresh- and marine sediment data pooled; freshwater midge data seen as relevant for the marine environment. -Freshwater midge considered different enough in life history from 2 marine chronically tested species (that have similar living and feeding strategies). -Sub-lethal endpoint from most sensitive acute test used as supporting information. -No long term test for most sensitive species in acute studies. -Field study results seen as equivocal in terms of assessment factor adjustment.	CTT are not proposing a "near field" EQS because this derivation is not covered by CIS 27. See discussion in sediment EQS section of the report.	Lowest relevant & reliable study (96-hour *Americamysis bahia* LC50 0.078ug/l); assessment factor 10 -freshwater and saltwater data pooled. -assessment factor as recommended by CIS 27.	Lowest relevant & reliable study (28d *Americamysis bahia* EC10 9.44ng/l); assessment factor 50 -freshwater and saltwater data pooled. -assessment factor as recommended by CIS 27.

dwt – dry weight; *wet weight standards

CTT have not recommended an EQS for "near field" sediment effects because this is a regulatory construct used in SEPA's regulation of fish farms, not a situation that is covered in CIS 27; it represents more of a trigger value for monitoring requirements than a protection goal in its own right.

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Introduction

Chemistry Task Team (CTT) of the UK's Technical Advisory Group (UKTAG) was asked to recommend environmental quality standards (EQS) for the fish farm sealice medicine emamectin benzoate.

The Scottish Environment Protection Agency (SEPA) previously set standards for the substance in 1999, before current EQS development guidance and significant new data were available.

In forming their opinion CTT considered several key pieces of information:

i. an EQS report commissioned by SEPA and produced by the environmental consultancy WRc in January 2017 (WRc 2017);
ii. the comments of three independent peer reviewers on the WRc report;
iii. a 2017 SEPA field study conducted in the Shetland isles, including one of the peer reviewer's comments on this (SEPA 2018);
iv. a recent industry-funded fish farm field survey carried out in 2017
v. results of more recent ecotoxicity testing conducted by Industry

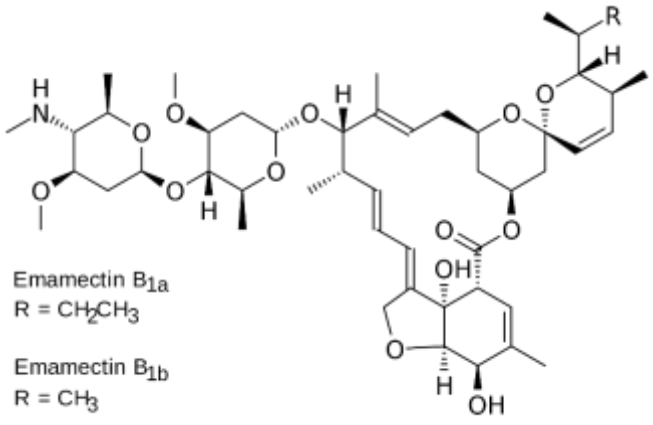
The above information is summarised and discussed below, followed by CTT's opinion.

An industry-sponsored EQS derivation report that includes the new ecotoxicity data and industry field data was also submitted as part of the data package. CTT discuss this derivation in a separate background document.

Background to the substance

The information in this section has been summarised from WRC 2017 and the 2011 Draft Assessment Report for the substance, carried out under the EU Plant Protection Products Regulation (EC, 2011).

Emamectin benzoate is a mixture of emamectin B1a ((0%) and emamectin B1b (10%). Both isomers are large molecules (ca. 1000 RMM) and differ by one methyl group (benzoate molecule not shown):



The substance is a white solid at room temperature with a melting point of 160 degrees C and a low vapour pressure. Its water solubility is 24 mg/l at pH 7. The substance contains two functional groups that are ionisable at environmentally relevant pH. Consequently, the substance's octanol-water partition coefficient varies between 3 and 5.9 in the pH range 5 – 9, with a log Kow of 5 at pH 7. Emamectin benzoate is hydrolytically stable between pH 4 and 8. In three studies of varying experimental design, it had measured DT50s for direct photolysis of 32 – 65 days; 22.4 days in phosphate buffer and 6.9 days in natural pond water; and 0.89 days in buffer. (Further details of these studies were not reviewed as photolysis is an unlikely degradative pathway based on the substance's environmental release from use in fish farms). In an OECD 301F (manometric respirometer) study emamectin benzoate was not readily biodegradable. Two simulation studies are available in sediment/water systems, adhering to the general principles of OECD 308. The Draft Assessment Report deemed the first not reliable because of issues around exposure. The second study reported a DT50 in water for dissipation of 8.7 days. This value will largely reflect the partitioning of the substance to the sediment. DT50 for sediment was reported as >120 days, with the overall DT50 for the system >120 days. Formation of metabolites and mineralisation were very low, with radioactivity being mainly associated with the parent substance and bound residues at test end. Although the octanol-water partition coefficient indicates the potential for aquatic bioaccumulation, emamectin benzoate has a low measured steady state BCF of 82 L/kg in bluegill fish (*Lepomis macrohirus*).

No harmonised EU classification is available for emamectin Benzoate.

The only known current use of emamectin benzoate in the UK is as an in-feed medicine in finfish aquaculture to control sealice, e.g. *Lepeophtheirus salmonis*, in salmonids. The substance has a well investigated mode of action, involving binding to *gamma* aminobutyric acid receptors (GABA receptors) and glutamate gate chloride channels with subsequent disruption of nerve signals in arthropods (Wolstenholme 2012) and relevant for other taxa (Lynagh et al 2015).

Summaries of available information

i. Summary of the 2017 WRc EQS proposal Report (WRc 2017)

This report, commissioned by SEPA and available on SEPA's website, was produced to review additional ecotoxicity data that had become available since the previous (1999) setting of SEPA standards for the substance and to propose EQS based on the EU Water Framework Directive (WFD) EQS guidance (the 2011 WFD Common Implementation Strategy technical guidance 27; herein CIS 27). The report identifies the key QS; in terms of regulating substances that behave like emamectin benzoate that are used as in-feed fish farm treatments, this is generally the EQS set for the protection of sediment dwelling organisms based on exposure considerations. The report derives two marine sediment QS, one protective of "near field" effects and the other protective of "far field" effects, in line with the approach taken to regulating the substance in Scottish fish farms. In addition, EQS are derived for marine water (protective of pelagic organisms), both for long term ("annual average", termed AA-EQS_{water} in this paper) and short term exposures (a maximum allowable concentration, termed MAC-EQS_{water} in this paper). The report makes a case for combining the freshwater and saltwater ecotoxicity data on the basis of no obvious differences in sensitivities and knowledge of the substance's toxic mode of action.

Both EQS for sediment were derived based on the only available chronic sediment toxicity study at the time - a freshwater sediment dwelling chironomid (midge) species. The far field EQS uses an assessment factor (AF) of 100 whereas the near field uses an AF of 10 on the same no observable effect concentration (NOEC) endpoint (emergence). The EQS for acute effects in pelagic organisms is based on an acute toxicity study in mysid shrimp with an AF of 50, while the EQS for chronic effects uses a chronic study in the same species and an AF of 20.

ii. Summary of Peer Reviewer comments on WRc (2017) report

SEPA coordinated a peer review of the 2017 WRc EQS report. Three independent peer reviewers were posed the following five questions:

1. *Has the EQS been correctly and appropriately derived in light of the available information and following the WFD guidance document No. 27 for deriving EQS?*
2. *Using the data available, has the most critical EQS been correctly identified in the context of impacts on benthic fauna?*
3. *Using the available data, can saltwater and freshwater ecotoxicity data be pooled for EQS derivation in this instance? If so, is the use of freshwater sediment insect data a valid approach for setting a marine sediment EQS for the protection of benthic fauna in this case.*
4. *Are the key data used in the EQS derivation reliable and relevant?*
5. *Is the method adopted for the proposed EQS better than that used to derive the current EQS for the long-term protection of marine benthic fauna?*

The paragraphs below summarise the peer reviewers' responses to the questions.

1. *Has the EQS been correctly and appropriately derived in light of the available information and following the WFD guidance document No. 27 for deriving EQS?* One reviewer felt some AFs were overly stringent based on knowledge of the mode of action, and recommended lowering these AF. The second peer reviewer also questioned AF selection, stating it was not in-line with the guidance for some EQS. Both of these peer reviewers, for the far field sediment EQS, recommended using further justification for the AF selection; the first reviewer suggested an AF of 10, the second sought further justification for the WRc proposal of an AF of 100 (which they thought was too low in the absence of further justification, in contrast to the other reviewer). Peer reviewer 2 recommended using a different study for the AA-EQS_{water} and increasing the AF to 100 from 20. This reviewer also stated a case could be made for lowering the MAC-EQS_{water} to 10 (from 50). Peer reviewer 3 did not question AF selection.

2. *Using the data available, has the most critical EQS been correctly identified in the context of impacts on benthic fauna?* The peer reviewers seemed to think that it had. It is implied that no other approach is really possible, although additional evidence should be used to justify what has been presented (see above).

3. *Using the available data, can saltwater and freshwater ecotoxicity data be pooled for EQS derivation in this instance? If so, is the use of freshwater sediment insect data a valid approach for setting a marine sediment EQS for the protection of benthic fauna in this case?* The peer reviewers all felt that pooling the pelagic data was justified and the correct approach in this case based on the available data. In addition, one reviewer reworked the statistical analysis to demonstrate this, although the dataset

was stated to be rather limited for statistical manipulations. All peer reviewers felt using the freshwater sediment data was appropriate for QS setting in the marine environment in the absence of marine data.

4. *Are the key data used in the EQS derivation reliable and relevant?:* reviewers generally felt more evidence was needed on the reliability of the two saltwater studies used to derive the two pelagic EQS, since the test reports were not available for scrutiny. One suggested the other test data could be used in a weight of evidence approach to support inclusion of these studies to derive the EQS.

5. *Is the method adopted for the proposed EQS better than that used to derive the current EQS for the long-term protection of marine benthic fauna?:* of the reviewers that commented, the new derivation in line with current guidance was seen as an improvement.

Other significant comments: Two reviewers recommended that a weight of evidence approach be taken i.e. looking at the different lines of evidence of this chemical's toxicity, to further justify AF selection for the sediment far field QS (see question 1 summary above). These lines of evidence should include:

(a) equilibrium partitioning to estimate chronic sediment toxicity from the AA-EQS_{water};
(b) an acute marine sediment study to derive a long term QS; and
(c) field data

Peer reviewer 1, aware that further testing is being undertaken, believed on balance that the EQS should be put on hold until new test data is available.

iii. Field data: SEPA study

Based on sampling transects of eight fish farms with "matched" benthic fauna and chemical sediment residue analysis (86 data points) in early 2017 in the Shetland Isles, SEPA produced a summary of their generalised linear mixed modelling (GLMM) data analysis and Canonical Correspondence Analysis. GLMM analysis showed that emamectin benzoate concentration had by far the biggest effect on crustacean abundance and number of crustacean species (other parameters considered included total organic carbon, particle size, position relative to predominant flow direction and enrichment of polychaete abundance). The statistical analysis was independently reviewed by Biomathematics and Statistics Scotland (BioSS).

During the WRc report peer review, only one peer reviewer was able to consider the field data. This reviewer felt that the data, whilst indicating an effect on abundance and diversity, were not sufficient to derive EQS. Although further statistical analysis could help, they were unlikely to give an unequivocal result by themselves. Despite the high variability seen in the field data, the highly relevant nature of the data could be used to test whether the proposed EQS was likely to be over- or under-protective.

iv. Field data: Industry-sponsored Passive Field Monitoring Survey

Later in 2017 industry initiated a field study to look at concentrations of emamectin benzoate in marine sediments in the vicinity of fish farms in relation to indicators of impacts in benthic fauna. 19 fish farms in the Outer Hebrides, west coast, Shetland and Orkney were surveyed based on a consideration of historical Slice use. The parameters measured were similar to the SEPA study, although the study design seemed to differ in that most sampling stations were farther from cage edges (up to 10 stations per farm that included a reference

station as well as sites at multiple distances outside the fish farm cage edge, mostly beyond the allowable zone of effect, AZE).

As expected, emamectin benzoate concentrations were lowest farthest from fish farm cage edges and increased the closer the station was to the fish farm. Macroinvertebrate species diversity and abundance varied greatly across the survey, although an impact was demonstrated with the extremes of the data. Notably, species diversity and abundance varied greatly in samples with no emamectin detected above the limit of detection, presumably a product of the regional variability in environmental conditions of the various farms and regions surveyed.

The authors found an apparent relationship between emamectin concentrations and species richness as a decline in richness between the limit of detection and around 50ng/kg. Above this concentration no further impact was noted. As this relationship seemed unlikely (generally it should be possible to locate a "point of departure" and at concentrations above this observe steadily increasing impacts), the authors "truncated" the data to exclude concentrations less than 0.01 ug/kg and greater than 1 ug/kg wet weight for further statistical analysis, however this approach showed no relationship between species richness and emamectin concentration. The authors did find an apparent relationship between particle size and species richness, and less so for organic carbon and species richness, so environmental factors such as these will have contributed to the lack of clear conclusions from the study. Overall it seems that the inherent variability in the data, the effect of environmental factors, and potentially the survey design (widely spaced sampling points over a wide range of fish farms with different environmental characteristics, as demonstrated by the differences noted in less than limit of detection samples with respect to species present) contributed to the inconclusive findings of the study.

v. Recent ecotoxicity data

In addition to the dataset described in WRc 2017, the industry conducted further ecotoxicity testing in 2017 and 2018. Studies in marine pelagic and sediment-dwelling organisms were conducted and made available for CTT review, as follows:

- 96-hour acute toxicity of emamectin benzoate to the mysid shrimp *Americamysis bahia* (EPP 2018a).
- 28-day chronic toxicity of emamectin benzoate to the mysid shrimp *Americamysis bahia* (EPP 2018b).
- 10-day acute toxicity of emamectin benzoate to the lugworm *Arenicola marina* (EPP 2018c)
- 10-day acute toxicity of emamectin benzoate to the marine amphipod *Corophium volutator* (EPP 2018d)
- 28-day chronic toxicity of emamectin benzoate to the marine amphipod *Leptocheirus plumulosus* (EPP 2018e)
- 28-day life cycle toxicity of emamectin benzoate to the marine amphipod *Leptocheirus plumulosus* (EAG 2018)
- 28/75-day chronic toxicity of emamectin benzoateto the marine amphipod *Corophium volutator* (Scymaris 2018)

CTT opinion & recommendation

CTT's opinion on the derivation of EQS for emamectin benzoate, described below, has been organised into sections for each EQS. Each section begins with a summary of the available dataset followed by a description of CTT's approach to EQS derivation, justifying decisions made with reference to CIS 27 and the WRc (2017) report and its peer review where necessary. Based on the substance's use pattern, CTT have only derived EQS for the marine environment. Where applicable, CTT refer to the five questions posed to peer reviewers (see *summaries of available information* section). Field data are considered in relation to the sediment EQS. A table at the end of this paper summarises CTT's recommendations for EQSs for marine sediment and water.

CTT make this recommendation following CIS 27 guidance, which draws on guidance set under the REACH regulation (ECHA 2008), and use of expert judgement using the different lines of evidence available in this case. Where necessary data have been reviewed according to the principles of the CRED system that includes an assessment of test reliability, according to the Klimisch scheme, and relevance for the use to which the data is being put (review sheets for new ecotoxicity studies are available in the annex).

General notes on EQS derivation

There are currently two recommended approaches to EQS derivation, the so-called deterministic and probabilistic approaches. In the former, the key datapoint (ie lowest ecotoxicity result from a reliable and relevant study) in the compartment-specific ecotoxicity dataset is selected and an assessment factor is applied to it to account for uncertainties that include laboratory to field extrapolation, representiveness (unknown sensitivity of untested taxa), etc. The latter approach can be used for larger datasets, where a substance's toxicity profile has been better investigated through laboratory tests representing many taxanomic groups and species. In this approach a distribution of the sensitivities of tested species is plotted relative to common toxicity metrics (NOEC or EC10 for chronic toxicity studies) in a Species Sensitivity Distribution (SSD), and this is used to derive the concentration that is hazardous for 5% of the tested species (the HC_5). An assessment factor is applied to this HC5 to derive the EQS. The AF is lower than those used in the deterministic approach because levels of uncertainty are lower owing to the more extensive dataset.

Pelagic EQS

The technical guidance CIS 27 notes that if no systematic or statistical differences are apparent between marine and freshwater data, then it is appropriate to pool datasets in EQS derivation. The available data and statistical reanalysis by a peer reviewer identified no differences between the fresh-and saltwater datasets. In line with CIS 27, CTT believe pooling the available data is appropriate. CTT agree the apparent differences in the original statistical analysis are probably a consequence of the differences in species composition, with a much higher % of sensitive taxa in the marine dataset (see peer review summary above).

MAC-EQS$_{water}$

The available reliable and relevant dataset includes:

- Marine: acute toxicity in 7 crustacean (1 lobster, 2 shrimp and 4 copepod species), 1 mollusc and 1 fish species
- Freshwater: acute toxicity in 1 algal, 1 crustacean, 1 insect and 4 fish species

There are not enough data to satisfy CIS 27's requirement for the construction of a species sensitivity distribution to use a probabilistic EQS derivation, so the deterministic approach is used. In the original dataset (see WRc 2017) two acute mysid shrimp (*Americamysis bahia*) studies with the same result were available (96h LC50 0.04ug/l). This was the most sensitive species in the dataset. One of these studies was used as the critical datum to set SEPA's existing QS in 1999, and on this basis, in the absence of further information on the study, WRc (2017) stated it was reliable and used it as the critical datum in their EQS derivation. The available information for these studies (cited as WRC 2000, and EFSA 2009[1] and Environment Canada 2005[2] in WRc 2017) point to the same source (USEPA's Office of pesticide Program, 2000), so this may in fact be the same study. No further information than that in WRc 2017 is available, nor is further detail on the previous SEPA review available. One peer reviewer commented that despite these issues the other available data could still support the test's use as the basis for the EQS. However CTT feel that the variability in the dataset prevented this conclusion. CTT therefore feel that the study (or both studies) should be reliability 4 (unassignable), not 2 (reliable with restriction). Recently Industry conducted a repeat mysid shrimp acute toxicity study (EPP, 2018) according to OPPTS Guideline 850.1035: Mysid Acute Toxicity Test (1996). This gave a 96h LC50 of 0.078ug/l. This result is the lowest L/EC50 in the acute dataset. CTT has reviewed the test report and deem the study reliable and relevant (reliability 2, reliable with restrictions based on some issues with test solution analysis and lack of a test concentration causing significantly >50% mortality, meaning that the LC50 is slightly extrapolated in the dose-response curve). For the reasons stated CTT does not think it is appropriate to use the other two mysid shrimp studies in EQS development (ie not appropriate to use a geometric mean of the studies, as CIS 27 recommends when there are multiple reliable studies for the same species tested following similar protocols).

In the WRc (2017) report peer review, one reviewer suggested additional data for the crustacean Norway lobster (reliability 2, reliable with restrictions) could justify lowering the assessment factor to 10, because this species is significantly different from the other crustacean (copepods), having a different feeding strategy. CTT agrees with this approach and believe there are enough reliable and relevant studies in the dataset to justify the use of an assessment factor of 10. To use this assessment factor CIS 27 states "*at least one short-term L(E)C50 from each of three trophic levels of the base set (fish, crustaceans and algae) + two or more short-term L(E)C50s from additional specific saltwater taxonomic groups*", and "*known mode of toxic action and representative species for most sensitive taxonomic group included in data set*"). Base set taxa (algae, invertebrates, fish), at least two acute studies in additional marine taxa (Norwegian lobster and a mollusc species) are available, the substance's mode of action is understood, and sensitive taxonomic groups are included in

[1] https://www.efsa.europa.eu/en/efsajournal/pub/rn-290 Note as one peer reviewer commented this appears to be the wrong reference.

[2] http://publications.gc.ca/collections/Collection/En4-51-2005E.pdf

the dataset (ie crustaceans with GABA receptors). Taking the lowest acute result ie the new mysid shrimp LC50 of 0.078ug/l, gives a **MAC-QS_{water} of 0.0078 ug/l, or 7.8 ng/l**.

AA-EQS_{water}

Available reliable and relevant dataset:

- Marine: longterm toxicity in 2 crustacean species
- Freshwater: longterm toxicity in 2 primary producers (algae and lemna), 1 crustacean, and 1 fish species; freshwater microcosm study

There are not enough data to satisfy the TGD's requirement for the construction of a species sensitivity distribution to use a probabilistic EQS derivation. The lowest chronic effects data (28-day mysid shrimp NOEC (growth) 0.0087ug/l) were used in the WRc (2017) derivation of the AA-QS_{water} with an AF of 20 to give an AA-QS_{water} of 0.435ng/l. However peer reviewers of the WRc report highlighted issues with the reliability of the data. One peer reviewer felt that the uncertainties with this test meant it should not be used. Since the WRc report and its peer review, an additional study has become available, a repeat 28-day mysid shrimp chronic toxicity study (EPP 2018b) based on US EPA guideline OPPTS 850.1350 (1996) that reported an EC10 of 9.44ng/l for reproduction.

CTT agrees with the peer reviewer comments that the original mysid shrimp study should not be used. Attempts to locate more detail on the mysid shrimp study led to the same conclusion as for the acute study – it should be reliability 4 (unassignable) as there is not enough information to judge whether it is reliable or not. CTT has reviewed the new mysid shrimp study report and deem the study reliability 2, reliable with restrictions (similar issues around test substance exposure concentration validation as for the new acute study). The lowest NOEC/EC10 was for female body length with a NOEC of 4.13ng/l. This value was derived at a significance level of 1%, not 5% as is more usual, and no EC10 could be derived for the effect below the highest concentration tested. There was no effect for the same endpoint for males. As it is unlikely there would be a sex-specific growth difference, the result is uncertain and has not been used here. The next lowest NOEC was 7.84ng/l (EC10 24.52ng/l) for mortality in the G2 generation at day 28. However, this is not stated to be a key endpoint for this test as the EPA do not recommend an MATC is developed for it. The third lowest endpoint was an EC10 of 9.44ng/l (95% CI 1.72, 15.01) for reproduction (offspring per surviving female per reproduction day); a NOEC of 17.07 was given for the same endpoint. (See annex for CTT study review details). The results for this endpoint are also close to those for mortality in the G2 generation. On balance, CTT thinks that the EC10 of 9.44ng/l for reproduction is the key endpoint to take forward for hazard assessment.

The dataset includes four reliable chronic studies in freshwater organisms (3 taxa) in addition to the new mysid shrimp and the *Acartia clausi* marine studies (the oyster studies include in WRc 2017 are considered sub-lethal, not true chronic studies). A freshwater microcosm study with a community NOEC of 0.1ug/l is also available. CIS 27 states that an assessment factor of 50 applies when there are:

> *Two long-term results (e.g. EC10 or NOEC) from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) plus one long-term result from an additional marine taxonomic group (e.g. echinoderms, molluscs)*

and 10 for:

> *Lowest long-term results (e.g. EC10 or NOEC) from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels + two long-term results from additional marine taxonomic groups (e.g. echinoderms, molluscs)*

The additional marine studies have to represent taxa with different living/feeding strategies to those of the organisms used in "core" chronic studies. In this case, CTT believes this difference is not marked enough for the copepod species, so based on the additional marine study an assessment factor of 50 applies. Applying this to the lowest available result, the EC10 for reproduction from the recent mysid shrimp study, gives a **AA-QS$_{water}$ of 0.19 ng/L**.

Sediment EQS

SEPA use the concept of a "far field" and "near field" sediment EQS in their regulation of fish farms. The far field EQS is the situation covered by CIS 27, equivalent to an "annual average" water EQS (protective of chronic effects in sediment dwelling organisms on the basis that sediment exposure is likely to be long lived, especially in the case of persistent substances). It is used in regulation for compliance assessment. The near field EQS seems to be used in regulation as a trigger for additional far field monitoring requirements, and so could be thought of as more like a MAC (maximum acceptable concentration), although for the reasons stated MAC are less relevant for sediment exposures to substances of this kind. As this is a non-standard endpoint, CTT have focussed on the derivation of a sediment EQS in line with the principles of CIS 27.

There are not enough data to distinguish any differences in sensitivities between freshwater and marine sediment-dwelling organisms. As is the case for the pelagic data, CTT has followed CIS 27 guidance and pooled fresh- and saltwater data. This is further discussed below in relation to relevance.

Available reliable and relevant dataset:

- Marine: longterm toxicity in 2 crustacean species (3 studies in 2 copepod species); sub-lethal endpoint from acute toxicity study in a polychaete species (the lugworm *Arenicola marina*)
- Freshwater: longterm toxicity in 1 insect species

The only reliable chronic sediment study available to WRc (2017) and subsequent peer review was the 28d emergence test with the freshwater midge *Chironomus riparius*. The peer reviewers agreed it was reasonable to use this freshwater study to derive a marine sediment EQS, following CIS 27 guidance. Since then three additional industry-generated chronic studies became available, two in the marine amphipod *Leptocheirus plumulosus* (EPP 2018e; EAG 2018) and one in the marine amphipod *Corophium volutator* (Scymaris 2018). In addition, the industry conducted an additional acute toxicity study in the lugworm *Arenicola marina (EPP 2018c)* and an acute toxicity study in the same corophium amphipod species (EPP 2018d) as the chronic study. The chronic corophium study included the more usual 28-day duration results but was also continued to day 75. The new studies all followed accepted international or national (US EPA) guidelines except for the chronic corophium study, the protocol for which was based on well document literature sources. Of the four available chronic studies, the most sensitive is the freshwater midge study (28 day NOEC 1.175 ug/kg dwt). Considering the marine data in isolation, the sub-lethal endpoint in the

new acute Arenicola lugworm study gave a lower result than those observed in the three marine chronic studies. This is a 10-day EC10 for casting of 12.9ug/kg dwt (the lowest endpoint from the chronic studies is the geometric mean for the EC10 for growth rate from the two leptocheirus studies, 30.5ug/kg dwt).

CTT has reviewed the three additional chronic marine sediment studies and the two additional acute sediment studies and finds them all to be reliable and relevant, appropriate for use in hazard assessment and EQS derivation (see annex). However it should be noted that the sub-lethal endpoint from the acute arenicola study has some shortcomings as it seems to be inherently linked to mortality, with this relationship having a greater impact at higher concentrations. The endpoint is based on the total number of casts, recorded daily in the 10-day test, but the decreasing number of animals in test concentration vessels is not taken into account in the statistical analysis. Reanalysis of the data to make this correction does not seem possible based on study design (ie not possible to count surviving worms at the same frequency as casts). Whilst the EC10 for casting (12.9 ug/kg) is lower than the NOEC for mortality (19.9 ug/kg), correcting for mortality would affect the slope and shape of the dose-response curve and so likely influence the casting summary statistic value. Nevertheless, the results seem to indicate an effect is occurring such that the results are not solely driven by the decreasing number of worms.

CTT also considered the available acute sediment toxicity dataset, because the lugworm result for mortality (LC50) indicated that the most sensitive species in acute studies may not have been tested in longterm studies. Reliable acute studies are available in:

- *Arenicola marina*: 2 studies 10-day LC50s 111ug/kg & 40.8ug/kg
- *Corophium volutator*: 2 studies 10-day LC50s 193ug/kg & 141 ug/kg[3]
- The spot prawn *Pandalus platyceros*: 8d EC20 (mortality) 138ug/kg

It can be see that the most sensitive species was the lugworm *Arenicola marina*, however two amphipod species have been used for chronic testing rather than this or a related species. Of these amphipod tests, the two *Leptocheirus plumulosus* chronic studies showed effects whereas the *Corophium volutator* did not. According to CIS 27 in the selection of assessment factors, chronic test data should cover the most sensitive species in the available acute studies.

In deriving an EQS for sediment in this situation, there are three main factors to consider:

i. selection of the key study and endpoint depending on reliability and relevance; the key consideration in this case is the relevance of the freshwater midge data to the marine environment now that marine test data are available
ii. the appropriate assessment factor based on the completeness of the dataset and;
iii. how additional lines of evidence (e.g. field studies, acute dataset, investigated mode of action) affect the choice of assessment factor

In addition, for studies in sediment, it also needs to be considered whether normalisation of the data to a set organic carbon content fraction is appropriate both for comparison of studies and final EQS setting.

The flow chart below (figure 1) has been compiled to illustrate how different decisions on the first two bullets above based on different interpretations of the data and CIS 27

[3] A sediment-free corophium study is also available but deemed not relevant

guidance can affect the outcome in this case (without consideration of organic carbon content).

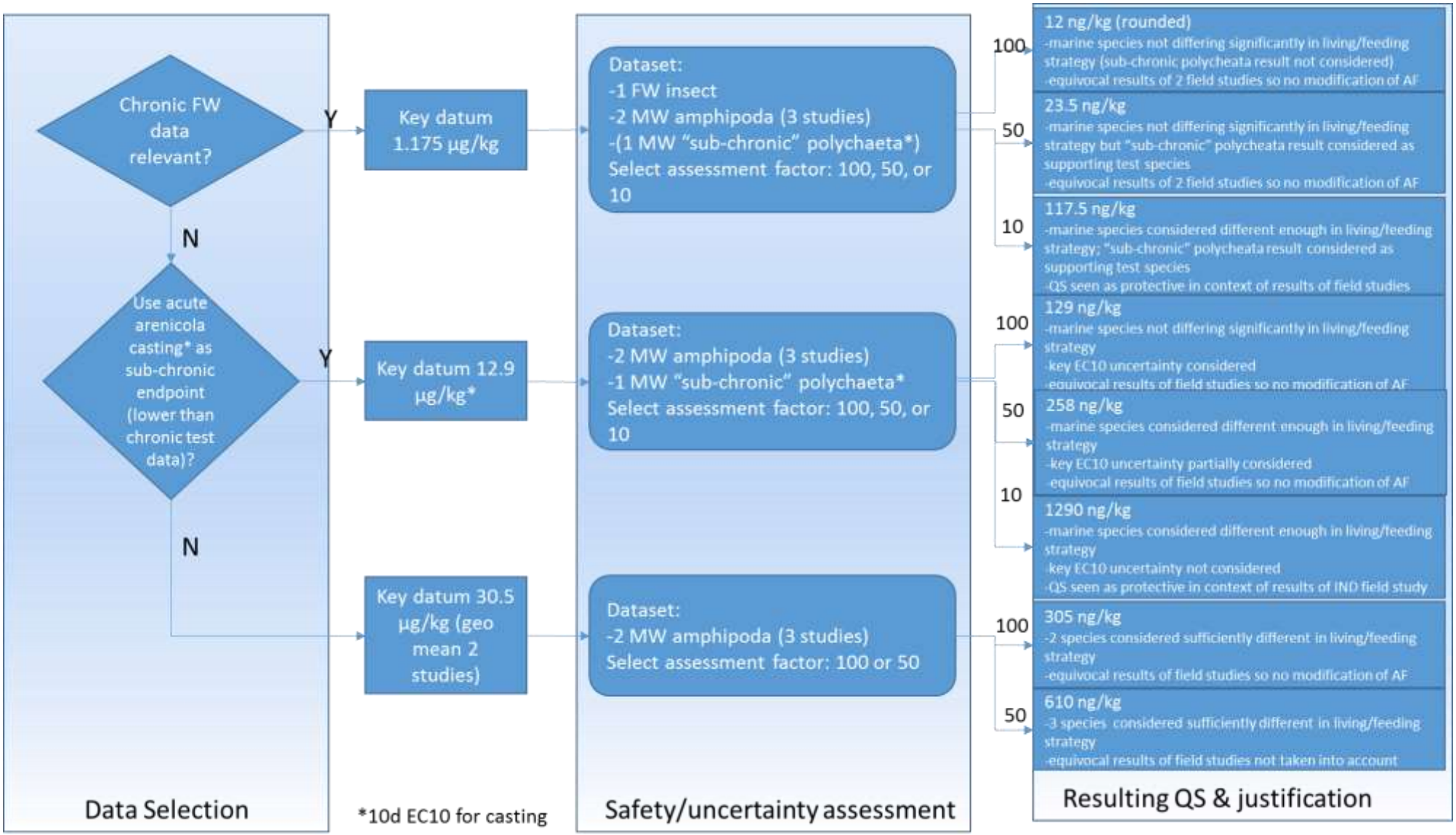


Figure 1: The impact of key data selection and assessment factor selection on sediment QS derivation

i. Key data selection

As stated above, based on the lack of obvious differences in sensitivity in the freshwater and marine datasets, the WRc (2017) report and the peer reviewers of the report decided that pooling of freshwater and marine data was acceptable for pelagic EQS development in line with CIS 27 guidance (CTT agrees with this). Based on this decision and the lack of additional chronic data in sediment dwelling organisms, they also decided that the chronic freshwater midge emergence study was appropriate for sediment EQS development. CTT also agrees with this, but given the new studies in marine organisms an assessment of the relevance of freshwater insect species for the marine environment is necessary (note there are not enough data to assess relative sensitivities of freshwater and marine sediment dwellers). Although very rare, insects with interdidal/marine aquatic larval stages are known in the UK. According to Langton and Pinder (2007) in Britain there are almost 600 species of non-biting Chironomidae midge, in addition to 161 species of biting midges of the Ceratopogonidae family (Chandler 1998). Whilst the majority of these species inhabit freshwater rivers, streams and ditches as well as brackish water, the larvae of *Clunio marinus* inhabit fully marine waters, being most abundant in the mid-littoral zone. This species has been surveyed in the west of Scotland (O'Reilly 2008). Most of this species' life history is associated with the sediment, with adults emerging and reproducing in a matter of hours before both adult males and females die without feeding. Therefore insect data do seem relevant for the marine environment in this case. Further, given the inherently greater level of uncertainty in hazard assessment for the marine environment compared with the freshwater environment based on the greater number of (untested) taxa, a more precautionary approach can be justified. This is in keeping with the principles of CIS 27. In terms of exposure, many fish farms are situated in sea lochs or coastal waters that are protected from the rigours of the open sea; hence they are almost always in tidal zones such that sediment exposure to fish faeces deposition or other releases from the cages can occur both up- and down-gradient. This means that sediment exposure can occur in areas between cages and the shoreline, not just in areas between cages and the open sea.

Based on these considerations CTT believes that the freshwater chironomid data are relevant for marine sediment EQS development.

ii. Appropriate Assessment Factor

The available updated reliable and relevant chronic dataset includes studies in three species as follows:

- 28-day chronic toxicity to freshwater midge *Chironomus riparius* (WRc 2017)
- 28-day chronic toxicity to the marine amphipod *Leptocheirus plumulosus* (EPP 2018e)
- 28-day life cycle toxicity to the marine amphipod *Leptocheirus plumulosus* (EAG 2018)
- 28/75-day chronic toxicity to the marine amphipod *Corophium volutator* (Scymaris 2018)

In addition to these four studies in three species of arthropod, the 10-day acute toxicity to the lugworm *Arenicola marina* (EPP 2018c) study included a sub-lethal endpoint (EC10 for casting; see above discussion).

CIS 27 does not cover this exact situation. In table 5.3 CIS 27 provides guidance on the AFs to be applied depending on the dataset available:

- "*one long term freshwater and one saltwater sediment test representing different living and feeding conditions*" leads to an assessment factor of 100;
- "*three long term sediment tests with species representing different living and feed conditions*" gives an assessment factor of 50 and
- "*three long term tests with species representing different living and feeding conditions including a minimum of two tests with marine species*" leads to an assessment factor of 10.

The guidance to marine sediment assessment factors in general also states:

> "*The general principles of notes (c)[4] and (d)[5] as applied to data on aquatic organisms (Table 3.3) shall also apply to sediment data. Additionally, where there is convincing evidence that the sensitivity of marine organisms is adequately covered by that available from freshwater species, the assessment factors used for freshwater sediment data may be applied. Such evidence may include data from longterm testing of freshwater and marine aquatic organisms, and must include data on specific marine taxa*."

Despite the presence of an additional marine species, because this does not seem to represent a significantly different living and feeding condition, the "default" position would be to apply an assessment factor of 100 to the chironomid data, on the basis that the life history of the midge is significantly different to that of the marine amphipods (ie "different living and feeding conditions"). However, based on the increased confidence the additional study gives for toxicity in this taxa, the supporting sub-lethal effects data from the acute arenicola study, and the fact that the freshwater data represent a taxa known to be sensitive to the substance's mode of action, in keeping with the "general principles" guidance note above CTT believes that an assessment factor of 50 can be applied when considering the laboratory data in isolation.

iii. Additional lines of evidence

Additional lines of evidence can be used to modify assessment factors recommended for laboratory data through expert judgement. As described in the CIS 27 guidance, key information can relate to field studies. Peer reviewers of WRc (2017) also recommended QS development based on acute toxicity testing, either through the assessment factor approach using sediment dweller data or equilibrium partitioning approach using pelagic data, as further lines of evidence for choice of chronic data assessment factor. CIS 27 describes these approaches, in particular in relation to situations where no chronic data are available. Applying the assessment factor (deterministic) approach to the acute toxicity dataset available now would lead to a QS for sediment of 41 ng/kg dwt (rounded) based on the 10-day LC50 of 40.8 ug/kg in the lugworm (Arenicola). However CTT believes these are poor additional lines of evidence to inform choice of assessment factor for chronic data, since both are inherently less certain than chronic data; both approaches are often used to "drive" the need for chronic testing in risk assessment. The mode of action of emamectin benzoate appears to have been well studied, although a later publication appears to indicate it may be relevant for a wider range of species and taxa than thought previously (see Uses of the Substance section).

[4] Table 3.3 note c refers to situations where a standard assessment factor of 500 can be lowered
[5] Table 3.3 note c refers to situations where a standard assessment factor of 100 can be lowered

The best pieces of additional evidence that can be considered in relation to choice of assessment factor are the two field studies. Unlike laboratory toxicity data, such studies are usually high in relevance but low in confidence. Based on the results of statistical analysis for the SEPA study (SEPA 2018), no threshold for effects can easily be derived from these data. However the SEPA field study suggests that a concentration somewhere in the region 10 – 100 ng/kg dwt should be protective of impacts on macroinvertebrate abundance/diversity of benthic fauna. The industry-led field study gave quite different results, based in part CTT believes on the differences in study design (lower density of sampling points) and the way emamectin concentration ranges and species presence happened to fall in the analysed samples. Various statistical approaches were applied to the data, since initial analysis of the total dataset seemed to indicate a toxicologically implausible correlation between emamectin concentrations and species richness. Truncation of the concentration data allowed an investigation of the impact of concentrations in ranges representative of proposed EQS (see description of study). CTT believes the findings of the survey are equivocal because of the inherent differences in populations in samples, the noise in the data and lack of granularity in the sampling regime. Taking the results of both studies into account, CTT does not see a clear line of evidence that would enable a relaxing of the proposed assessment factor of 50, as discussed above.

Normalisation to a set organic carbon content (5% recommended in CIS 27): the freshwater chironomid study OC content was 4.5%. Because this content is close to the CIS27 guidance and the field study data show that sediment OC can vary greatly with distance from cage edge and tidal currents, CTT has not normalised the recommended sediment EQS to 5% OC.

Based on the currently available data and the considerations described above, CTT recommends applying an assessment factor of 50 to the chironomid data giving a **sediment EQS of 23.5 ng/kg dwt**.

"near field" sediment EQS

This derivation is not covered by CIS 27, as described at the start of this section. CTT have not proposed a value for this endpoint.

Although the near field EQS is described as being used to trigger additional monitoring in the far field for compliance assessment by SEPA, it is not clear how assessment factors, and so the relationship between the near field and far field EQS, were decided in derivation of the SEPA 1999 standards for which there is a factor of ten difference. In any case it is likely that relationships between "Allowable Zone of Effect" (ie the seabed area immediately impacted in a fish farm cage) concentrations and the "far field" EQS compliance will vary from farm to farm depending on specific issues related to the farm itself and environmental factors of the local area, many of which could be modelled. This adds complexity in that it seems likely that a single "near field" EQS that will ensure at all farms on the one hand adequate far field protection and on the other avoidance of wasted resources in unnecessary additional monitoring is challenging.

The original SEPA 1999 derivation used an assessment factor 10 times lower than that for the far field EQS. This appears a defensible approach for this non-standard endpoint, as it seems to represent a commonly accepted acute:chronic toxicity ratio if the "near field" EQS is considered a surrogate for a MAC.

Secondary Poisoning/exposure via the foodchain

Since the measured BCF of the substance is less than 100 (82 l/kg), it is not necessary to derive an EQS for secondary poisoning.

Summary of CTT recommendation

In the table below, CTT EQS recommendations along with justifications are summarised. For comparative purposes the SEPA 1999 standards are also presented.

	EQS			
	Sediment effects (ng/kg dwt)	Sediment "near field" effects (ng/kg dwt)	Pelagic acute effects (ng/l)	Pelagic chronic effects (ng/l)
SEPA 1999 standards	760 (rounded)*	7630*	n/a	0.2
CTT recommendation	**23.5**	**n/a**	**7.8**	**0.19**
CTT justification	Lowest relevant & reliable study (28-day *Chironomus riparius* NOEC 1.175ug/kg); assessment factor 50. -freshwater midge data seen as relevant for the marine environment. -Freshwater midge considered different enough in life history from 2 marine chronically tested species (that have similar living and feeding strategies). -Sub-lethal endpoint from most sensitive acute test used as supporting information. -No long term test for most sensitive species in acute studies. -Field study results seen as equivocal in terms of assessment factor adjustment.	Situation not covered by CIS 27. See sediment EQS section for discussion	Lowest relevant & reliable study (96-hour *Americamysis bahia* LC50 0.078ug/l); assessment factor 10 -freshwater and saltwater data pooled. -assessment factor as recommended by CIS 27.	Lowest relevant & reliable study (28d *Americamysis bahia* EC10 9.44ng/l); assessment factor 50 -freshwater and saltwater data pooled. -assessment factor as recommended by CIS 27.

dwt – dry weight; *wet weight standards; note these derivations are based on different datasets and different approaches

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EPP 2018b. Determination of chronic toxicity of emamectin benzoate to mysid shrimp (*Americamysis bahia*) (28-day flow-through).

EPP 2018c. Determination of acute toxicity of emamectin benzoate to lugworm (*Arenicola marina*) (10-day static).

EPP 2018d. Determination of acute toxicity of emamectin benzoate to marine amphipods (*Corophium volutator*) (10-day static).

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Annex: CTT review of industry-sponsored ecotoxicity testing conducted since 2017

1) 96h Mysid shrimp (*Mysidopsis bahia*) acute toxicity test

Test guideline: OPPTS 850.1035 (1996) (https://www.epa.gov/sites/production/files/2015-07/documents/850-1035.pdf)

Test reference: Determination of Acute Toxicity (LC50) of Emamectin Benzoate to Mysid Shrimp (*Americamysis bahia*) (96 h, Static), Test Facility Study No. EC 17081511, Report No. EPP00325, EPP limited, October 2018

General criteria	**Fulfilled?**
Test concentrations appropriate (<limit of solubility)?	Yes. Stock solution in acetone prepared, solubility (loading 50mg in 2.5mL) visibly assessed. Soluble up to ca 20mg/mL in acetone.
Test concentrations maintained if analytical verification employed?	**partially**. Measured concs at test start 68 – 94% of nominal in the five stock solutions, then used to calculate concs in the 30 exposure vessels (inc controls), so actual exposures not measured directly. Also only measured at test start, not test end. This means that exposure concentrations may have been over-estimated, as the substance is adsorptive, and the proportion adsorbed within the test system may have increased during the study as a static system was used. NB Original pre-study plan stated "samples of test solution from each test concentration will be taken for analysis at the beginning and end of the definitive test." Subsequent amendment resulted in what was done (appendix 1)
Test-specific criteria	**Fulfilled?**
One of two age classes used, either juveniles (<24h old) or young adults (5 – 6 d old)	Yes, <24h old juveniles. Range finder showed no difference in lifestage sensitivity
20 or more individuals per concentration, loading <30 mysids/L (static)	Yes. 800mL in 1L vessels each with 5 juveniles (4 x 5 = 20 mysids per conc). Loading ok.
5 or more concentrations in geometric series	Yes. 6.25 (4.96), 12.5 (10.05), 25 (21.74), 50 (34.01) and 100 (93.74) ng/L plus solvent cntrl and cntrl
2 or more replicates	Yes. 4 per treatment
(if applicable, preferred carriers = DMF, triethylene glycol, acetone, ethanol (no more than 0.1 mL/L)	Yes, Individual stock soln per test conc prepared and diluted. Acetone concs in final solns ok.

DO (60 – 105% sat), T (25 deg C, +/- 2 deg C), salinity (20 +/- 3 ppt), pH at start and end in each test chamber	Ok. See comment re temp. (22.8 – 24.4 deg C; pH 7.88 – 8.11; 81.7 – 96.1% sat DO; 20-21 $‰_0$ salinity) over all chambers.
<10% mortality in controls during test duration	Yes. Repeated definitive test 0% in solvent cntrl and cntrl.

Comments

Noted Study deviations: definitive test had to be repeated (pH and DO problems and >10% mortality in control). Temperature dropped below 23 deg C in some vessels, but only a minor deviation with no impact on survival.

GLP study. Test substance CAS 155569-91-8 95.6% pure. Static. 96h LC50 reported as 77.99ng/l (95% CI 51.41, 179.06 ng/l); NOEC 21.74ng/l.
Mortality in highest tested conc (93.74ng/l) at 96h was 3/5, 3/5, 3/5, 1/5, mean 2.5/5, ie 50%; would have been good to test above 50% mortality, but stats (inc plots) in annex look ok. Analytical method description ok.

Overall, given issues with test solution analysis and lack of a test concentration causing significantly >50% mortality (see emboldened text), study deemed reliability 2, reliable with restrictions.

2) 28 day Mysid Shrimp (*Americamysis bahia*) chronic toxicity test

Test guideline: OPPTS 850.1350 (1996) (https://www.epa.gov/sites/production/files/2015-07/documents/850-1350.pdf)

Test reference: Determination of Chronic Toxicity of Emamectin Benzoate to Mysid Shrimp (*Americamysis bahia*) (28 Day, Flow Through), Test Facility Study No. EC 17081512, Report No. EPP00326, EPP limited, October 2018.

General criteria	**Fulfilled?**
Test concentrations appropriate (<limit of solubility)?	Yes. Stock solution in acetone prepared, solubility (loading 50mg in 2.5mL) visibly assessed. Soluble up to ca 20mg/mL in acetone
Test concentrations maintained if analytical verification employed?	**partially**. Measured concs at test start and "expired" stock solns 78.4 – 92.6% of nominal in the stock solutions, then used to calculate concs in the 20 exposure vessels, so actual exposures not measured directly in exposure vessels. This means that exposure concentrations may have been over-estimated, as the substance is adsorptive. It is not possible to say to what extent measurement of expired stock solution would represent the proportion adsorbed within the test system during exposures (potentially a mitigating factor for this shortcoming). NB Original pre-study plan stated "samples of test solution from each test concentration will be taken for analysis at the beginning and end of the definitive test." Subsequent amendment resulted in what was done (appendix 1)
Test system	Flow through conditions, 5 vol replacements per day. Stock solutions made up weekly, fresh soln added to test system daily.
Test-specific criteria	**Fulfilled?**
juveniles (<24h old) ?	Yes
40 or more individuals per concentration in replicates of up to 8 individuals?	partially. 40 juvenile G1 in 2 retention chambers until d14, then distributed between 4 retention chambers per conc.
5 or more concentrations in geometric series?	Yes. 2.5 (2.02), 5 (4.13), 10 (7.84), 20 (17.07), 40 (37.05) ng/l + control + acetone control
2 or more replicates?	
(if applicable, preferred carriers = DMF, triethylene glycol, acetone, ethanol (no more than 0.1 mL/L)	Ok – see above.

DO (60 – 105% sat), T (25 deg C, +/- 2 deg C), salinity (20 +/- 3 ppt), pH measured weekly in each test chamber?	ok (22.6 – 24.9 deg C; DO 60.2 – 98.7% sat; salinity 18-21 $‰_0$)
<25% of F0 females fail to produce young/average number of young per female >3 per day in controls?	**OK.** As group housing used, not possible to assign offspring to a particular female (re <25% criterion). Average no. young criterion not fulfilled, however indications this criterion is difficult to achieve; OECD draft 2-gen mysid study uses alternative validity criterion of "average total number of young per control female in first two broods >8". This was fulfilled. (http://www.oecd.org/env/ehs/testing/OECD%20TG%20Mysid%202-gen_Draft%20for%20REVIEW_15%20July%202013.pdf) NB this study has not progressed since this draft and is currently off the OECD's test guideline development workplan.
Test conc measured on days 0, 7, 14, 21 and 28; mean measured conc used if variation >20% or mean measured <80% of nominal	See above
Comments	
Noted Study deviations: pH and temp deviations from protocol, however deemed minor so no effect on study parameters or results. Body lengths of G2 not recorded at test termination (not housed by age so would not have been possible to normalise results). Both fine. Test endpoints are: (i) (length of time for appearance of first brood); (ii) body lengths of adult males and females at sexual maturity and test end; (iii) cumulative young per female on day 28; (iv) cumulative dead adults on days 7, 14, 21, 28; (v) (if available, effects on G2 mysids (no. males and females, body length of males and females, cumulative mortality)). Reported endpoints were: (ii), (iii), (iv) and (v) (G2 mortality) GLP study, test substance purity 95.6%. reproduction in all test chambers began on d20. The lowest NOEC/EC10 was for female body length with a NOEC of 4.13ng/l. This value was derived at a significance level of 1%, not 5% as is more usual for tests of this type. Growth rate measured in male and female mysids but study reported a a NOEC for females of 4.13 ng/l and an EC10 for the same endpoint of > 37.05 ng/l (highest test concentration). Male growth rate NOEC and EC10 both > 37.05 ng/l. The basis for the effect in females seems to be only just statistically significant, and given that a difference in growth rates between males and females is highly unlikely, we think this is more due to	

biological variation/artefact of the test than a toxicologically significant finding. Therefore discount the female result for use in hazard assessment.
The next lowest NOEC was 7.84ng/l for mortality in the G2 generation at day 28. However, this is not stated to be a key endpoint for this test as the EPA do not recommend an MATC is developed for it (just endpoints (ii – iv above). The third lowest endpoint was an EC10 of 9.44ng/l (95% CI 1.72, 15.01) for reproduction (offspring per surviving female per reproduction day); a NOEC of 17.07 was given for the same endpoint. On balance, we think the EC10 of 9.44ng/l for reproduction is the key endpoint to take forward in hazard assessment.

Overall, given issues with test solution analysis (see emboldened text), study deemed reliability 2, reliable with restrictions.

3) 10 day static marine amphipod (*Corophium volutator*) acute toxicity test

Test guideline: OSPAR 2005 Part B (https://www.ospar.org/documents?d=6996) "…OSPAR agreed that, all tests already performed on sediment reworker species and offshore chemicals and oil-based muds should be accepted as long as they were carried out in accordance with the PARCOM Guidelines of 1991 regarding harmonisation of procedures of approval, evaluation and testing of offshore chemicals and drilling muds."
Test reference: Determination of Acute Toxicity (LC50) of Emamectin Benzoate to Marine Amphipods (*Corophium volutator*) (10 Day, Static), Test Facility Study No. EC 17081514, Report No. EPP00328, EPP limited, October 2018.

General criteria	**Fulfilled?**
Test concentration spiking technique appropriate?	Yes. Test substance ID and purity ok. Pre-study work on solubility ok. Stock solution in acetone prepared and 15g sediment spiked, then homogenised with bulk sediment (150rpm, 3hrs).
Test concentrations and analysis	Ok. 25, 50, 100, 200 and 400 ug/kg dwt, plus control and solvent control. Number and spacing adequate. Additional destructive replicate used for day 0 analysis. Analysis of exposure vessels in day 10. Mean measured concs as follows: 12.9, 46.1, 99.4, 186.7, 420.0ug/kg dwt.
Test organisms	Pre-study holding ok, size appropriate (>5mm) held in 48 litre vessels, 1L vessels used in test
Results sufficient for summary statistics	Yes. Mortality ranged 0 – 100% across exposure concs with derived dose-response curve as expected.
seawater	Obtained from the field and prefiltered
Test-specific criteria	**Fulfilled?**
Test sediment: muddy fine sand with 0.5 – 4% OC, silt/clay fraction (<63um) 5 – 20%, median grain size 90 – 125um?	Obtained from the field. Deviations from recommended values: low (0.32%) OC, silt/clay low (between 1 and 3%), median grain size high (183um). Based on control performance, deviations ok.
Minimum sed depth 15mm, sed:water ratio <0.2?	Ok. Sed depth 40mm, seawater added up to 850mL (70mm above sed surface).
>20 animals per conc, >2 replicates?	Ok. 2 replicates per conc, 10 animals per vessel (20 in total per conc).
DO (>85% sat), T (15 +/- 2 deg C), salinity (+/- 4), pH 7.5 – 8.5 measured at start, 24h, and two more times during 10 d duration, bar salinity (start and finish)	Ok. Measurements carried out in compliance (days 0, 1, 3, 7 and 10). All recommended ranges met in definitive test
NB No mention of control mortality during test (<10% in pre-test holding period)	Ok. During test 1 animal died in one replicate of each of the two controls (10%).
Comments	
Noted Study deviations: sediment characteristics deviated from those recommended.	

GLP study. Results: 10d LC50 based on mean measured concentrations 141.5ug/kg dwt. NOEC 99.4ug/kg dwt. No CI possible for the LC50 because of poor statistical fit of the data to the model. Part of this is a result of the chosen study concentrations. Significant mortalities occurred only in the two highest test concentrations, 200 and 400ug/kg dwt (nominal) only. This means the dose-response curve is trying to represent more of a step than a curve, hence the poorness of fit. The range finder employed concentrations of 1, 10, 100 and 1000ug/kg, with significant 10-d mortalities in the 100 (3/10) and 1000 (10/10) concentrations only, so the choice of 100, 200 and 400ug/kg dwt to cover these observations seems reasonable.

Overall, based on the slight deficiencies noted for the study it is deemed reliability 2, reliable with restrictions.

4) 10 day static lugworm (*Arenicola marina*) acute toxicity test

Test guideline: ICES Techniques in Marine Environmental Sciences Guideline No. 29 (https://www.ciimar.up.pt/hns/documents/guidelines/Thain_and_Bifield_2001.pdf)

Test reference: Determination of Acute Toxicity (LC50) of Emamectin Benzoate to Lugworm (*Arenicola marina*) (10 Day, Static), Test Facility Study No. EC 17081513, Report No. EPP00327, EPP Limited, October 2018.

General criteria	**Fulfilled?**
Test concentration spiking technique appropriate?	Yes. Test substance ID and purity ok. Pre-study work on solubility ok. Stock solution in acetone prepared and 16g sediment spiked, then homogenised with bulk sediment (150rpm, 3hrs).
Test concentrations	62.5, 125, 250, 500, 1000ug/kg dwt nominal; 19.9, 41.8, 110.8, 273.8 and 642.9 ug/kg dwt mean measured
Test animals	Ok. Field collected
seawater	Field collected and prefiltered
Test-specific criteria	**Fulfilled?**
Test sediment: muddy fine sand with 0.5 – 4% OC, silt/clay fraction (<63um) 5 – 20%, median grain size 90 – 125um?	Field collected, deviated from recommended OC low (0.2%), silt/clay fraction low (between 0 and 4%), median grain size high (282um). Based on control performance, deemed ok.
sed depth 30mm, 30mm water depth above sed?	Ok. Sed 40mm depth, seawater 120mm (total).
>20 animals per conc, >2 replicates?	No. only one replicate per conc. With 10 worms. However, test performance appeared ok. With controls not indicating unacceptably high mortality and a definite dose-response curve evident.
DO (>85% sat), T (15 +/- 2 deg C), salinity (+/- 4), pH 7.5 – 8.5 measured at start, 24h, and two more times during 10 d duration, bar salinity (start and finish)	Yes. Measured d 0, 1, 3, 7 and 10. All values within range.
<10% control mortality	Ok. 10% (1 worm) mortality in the solvent control only.

Comments

Test deviations: no replicates employed. Sediment characteristics deviate from those recommended, although of limited significance.

GLP study. Results: 10-d LC50 40.8ug/kg dwt, NOEC 19.9 ug/kg dwt. EC5, EC10 and EC50 for casting 8.7, 12.9 and 51 ug/kg dwt.

Mortalities counted at test end whereas casting counted daily. Total numbers of casts were 87, 83, 74, 43, 22.7 and 1 for the control, solvent control, and test concentration series. Casting activity seemed to decrease during the test, with an apparent drop off in controls after day 6. Total number of casts will have been affected by mortality, not just worms being moribund. Because mortality was only measured at test end (as is usually

only possible for tests of this design), it is not possible to correct casting results for mortality (ie total number of casts per worm) to give a truer measure for this sub-lethal endpoint. The key result in this study is the LC50 for mortality.

Overall, because replicates were not used in the study although a good dose-response curve fit was derived, it is deemed reliability 2, reliable with restrictions.

5) 28 day marine amphipod (*Leptocheirus plumulosus*) chronic toxicity test

Test guideline: EPA/600/R-01/020 (March 2001)
https://nepis.epa.gov/Exe/ZyNET.exe/30002GRK.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000004%5C30002GRK.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL

Test reference: Emamectin Benzoate: Marine Sediment Chronic Toxicity with Amphipod (*Leptocheirus Plumulosus*), Test Facility Study No. EC17081515, Report No. EPP00329, EPP limited, October 2018.

General criteria	**Fulfilled?**
Test concentration spiking technique appropriate?	Yes. Test substance ID and purity ok. Pre-study work on solubility ok. Stock solution in acetone prepared and 3kg sediment spiked, then homogenised with bulk sediment (150rpm, 3hrs).
Test concentrations	31.25, 62.5, 125, 250, 500ug/kg dwt nominal; 21.7, 51.7, 90.6, 235.1 and 444.8 ug/kg dwt measured d0; 8.6, 59.9, 84.6, 160.1, 277ugkg measured d28.
Test conditions	Semi-static exposure (pre-treated sediment), 2cm depth. Ca 400ml overlying water replaced 3x per week. 175ml sed, 725ml overlying water.
Test organisms	Cultured in house, all neonates.
Test-specific criteria	**Fulfilled?**
Test sediment: muddy fine sand with 1 – 7% OC, >5% silt, <85% clay	Deviations: OC low (0.32%), silt low (1%). Clay 3%. Median grain size 183um
>5 concs	Yes. See above
Neonates <24h old or size selected	Yes – see above. Size selected.
>20 animals per conc, >2 replicates	Yes. 4 replicates per conc, 20 animals per vessel
DO (>60% sat), T (25 +/- 2 deg C), salinity (5 or 20‰ +/- 2), pH 7 – 9	Yes. DO 91.3 – 100% sat, T 24.1-25.8 deg C; pH 7.31 – 8.71, salinity 20‰ Recorded at test start and then three times per week.
<20% control mortality (no single replicate >40%), measurable growth and reproduction in all control replicates	Fulfilled.

Comments

Deviations from protocol: sediment characteristics differ from those recommended, however this deosn't seem to have affected the test.

GLP study. Results based on day 0 measured concentrations, not mean measured (day 0 and day 28). Not clear why mean measured concentrations across the test duration were

not used. Dose-response curves presented and good fits for survival and growth rate. Poor fit for reproduction.
LC50 49.7ug/kg dwt (95% CI 44.2, 55.5; NOEC 21.7ug/kg); growth rate (mean weight per surviving adult) EC50 65.6ug/kg dwt (95% CI 58.9, 74.2; NOEC <21.7ug/kg); data scattering meant no EC values derived for reproduction (number of offspring per surviving adult; NOEC reported as 51.7ug/kg). Whilst NOECs and LOECs were reported, only EC50s and not EC10s were reported.
Reanalysis of the raw data in a separate EPP non-GLP report, still using measured concentrations from d0 only, gave an EC10 for growth rate of 17.6ug/kg dwt as the key endpoint from the study.

Overall, the study is deemed reliability 2, reliable with restrictions.

6) "Life cycle" 28 day marine amphipod (*Leptocheirus plumulosus*) chronic toxicity test

Test guideline: EPA/600/R-01/020 (March 2001)
https://nepis.epa.gov/Exe/ZyNET.exe/30002GRK.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000004%5C30002GRK.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL
Test reference: EMAMECTIN BENZOATE: A LIFE CYCLE TOXICITY TEST WITH THE MARINE AMPHIPOD (*Leptocheirus plumulosus*) USING SPIKED SEDIMENT, PROJECT NUMBER: 706A-104 MSD ANIMAL HEALTH INNOVATION GmbH STUDY NUMBER: S17199-00-SLI-ENV-OT, EAG Laboratories October 2018.

General criteria	**Fulfilled?**
Test concentration spiking technique appropriate?	Ok. High purity test substance, stock solution in acetone, serial dilution used, mixed with 70g sand, sand mixed with 630g dry sediment after solvent evaporated then homogenised.
Test concentrations	51, 130, 320, 800, 2000ug/kg dwt nominal; d0, 14 and 28 analysis. Mean measured over the study = 38, 100, 260, 670 and 1600ug/kg dwt.
Test conditions	Flow through (2 vol replacement per day). Pre-spiked sediment.
Test organisms	cultured
Test-specific criteria	**Fulfilled?**
Test sediment: muddy fine sand with 1 – 7% OC, >5% silt, <85% clay	Field collected. OC low (0.3%),
>5 concs	Ok. 5 concs
Neonates <24h old or size selected	7-8 days old.
>20 animals per conc, >2 replicates	Yes. 5 replicates, 20 animals each. Extra uninhabited replicates for water quality & chemical analyses
DO (>60% sat), T (25 +/- 2 deg C), salinity (5 or 20‰ +/- 2), pH 7 – 9	Slight deviation in temperature on days 9 and 10 of test, unlikely to have affected the study.
<20% control mortality (no single replicate >40%), measurable growth and reproduction in all control replicates	Slightly deviating. 80% survival in solvent control, 72% in control. Growth and reproduction observed in all controls.
Comments Deviations from protocol: GLP study. Results based on mean measured concs 28-d LC50 220ug/kg dwt (95% CI 180, 260ug/kg); LC10 75ug/kg dwt; EC10 male growth rate 57ug/kg dwt; EC10 female growth rate 49 ug/kg dwt; EC10 reproduction 43ug/kg dwt. Mean growth rate can be estimated at 52.85ug/kg dwt. NOECs and LOECs also reported for each	

endpoint, however given the mortality in controls in this case the ECx results may be more reliable. No dose-response curves presented in the study report.

Overall, the study is deemed reliability 2, reliable with restrictions.

7) 28/75 day marine amphipod (*Corophium volutator*) chronic toxicity test

Test guideline: none followed[6]. Test specific validity criteria related to the extended duration (75 days). Endpoints of survival and growth (length and weight) available after 28d; additionally, reproductive output (no. gravid females and neonates) available after 75 days (see last of the four references in footnote).

Test reference: Emamectin Benzoate: Determination of effects in a water-sediment system on growth and reproduction of *Corophium volutator* using spiked natural sediment, study number 1038.00101, Scymaris Ltd, October 2018.

General criteria	**Fulfilled?**
Test concentration spiking technique appropriate?	Test substance ID and purity ok. Serial dilution of a stock solution used to give test concentrations, spiked to sediment.
Test concentrations	20, 32, 100, 320, 1000, 3200, 10000, 32000 ng/kg dwt nominal. Analysis on days 0, 7, 28 and 75
Test conditions	Semi-static; weekly 80% overlying water renewal.
Test organisms	Field collected, animals (offspring) used in study <7 days old.
Water chemistry – range and variation in pH, temp, DO, salinity	
sediment	Field collected
replication	4 replicates per conc and control; half for d28 measurement, other half for day 75 measurement
Test specific criteria	
Control mortality <40% during test	Criterion fulfilled for day 28 results, not day 75.
Control animals should have mean length >4mm	fulfilled
Mean no neonates per control replicate >100 at test end	Fulfilled
DO >60% sat	Fulfilled

[6] Test method adapted from:
Brown R.J. Conradi M. and Depledge M.H. (1999) Long-term exposure to 4-nonylphenol affects sexual differentiation and growth of the amphipod *Corophium volutator* (Pallas, 1766). Science of the Total Environment, 233, 77-88.

(2) Scarlett A., Rowland S.J., Canty, M., Smith, E.L. and Galloway T.S. (2007). Method for assessing the chronic toxicity of marine and estuarine sediments- associated contaminants using the amphipod *Corophium volutator*. Marine Environmental Research, 63, 457-470.

(3) Heuval-Greve M., Postma J., Johan J., Koomann H., Bubbeldam M., Schipper C. and Kater B. (2007). A chronic bioassay with estuarine amphipod *Corophium volutator*. Test method description and confounding actors, Chemosphere, 66, 1301-1309.

(4) Fox M., Ohlauson, C., Sharpe, A.D. and Brown R.J. (2014). The use of a *Corophium volutator* chronic sediment study to support the risk assessment of medetomidine for marine environments, Environmental Toxicology and Chemistry, 33(4), 937-942.

Comments
Study conducted to generally accepted principles of sediment ecotoxicological study. GLP laboratory
Chemical analysis for emamectin conducted by a separate laboratory so results not to GLP. Maximum test concentration reported as 61282ng/kg dwt, mean measured, equivalent to the nominal concentration 32000ng/kg dwt.

Animals were assessed for survival, growth (length and weight) and reproductive output at days 28 and 75. Significant control mortality (47%) was observed at day 75, so results for this extended time period should be treated with caution. Overall, no effects were observed in the study for any endpoint for both durations, ie all NOECs were equal to the highest tested concentration.

On balance, the study is deemed reliability 2, reliable with restrictions.