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ECOTOXICOLOGY LABORATORY:
TECHNICAL JUSTIFICATION FOR THE SELECTION OF BIOASSAYS FOR A BITTERNS DIRECT TOXICITY ASSESSMENT (DTA)

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INTRODUCTION

The objective of this technical justification report is to investigate and determine the most appropriate ecotoxicological methods available for a direct toxicity assessment (DTA) of a hypersaline bitterns sample for a proposed solar salt field for Leichhardt Salt Pty Ltd (Leichhardt).

Leichhardt is proposing to construct and operate the Eramurra Solar Salt Project, a solar salt project with an annual average production capacity of 5.2 million tonnes per annum (Mtpa), of high-grade salt (sodium chloride (NaCl)) from seawater. The salt will be produced using a series of concentration ponds and crystallisers with a processing plant, transport corridor, stockpiling and export from the Cape Preston East Port. The concentration ponds and crystallisers will be located on mining leases.

The export of salt is proposed via a trestle jetty and bitterns will be transported by pipeline attached to the trestle jetty structure and discharged via a diffuser located off the trestle jetty. The Eramurra Solar Salt Project is located in the western Pilbara region of WA, approximately 55 km west-southwest of Karratha.

To assess the environmental impact of the bitterns on the local marine environment a DTA was undertaken to calculate safe dilution factors associated with the bitters discharge by using a species sensitivity distribution (SSD) following the methods detailed in the Australian & New Zealand guidelines for fresh & marine water quality (ANZECC & ARMCANZ 2000, ANZG 2018) and Warne et al. (2018).

The ANZG (2018) provides a framework to assess water quality using multiple lines of evidence in a weight of evidence framework. Direct toxicity testing is one line of evidence that provides strong evidence to be used to make management decisions of a discharge. DTA, also referred to as Whole effluent toxicity (WET) testing, is a well-established approach to assess the toxicity of a sample which is a mixture of contaminants and/or physiological stressors. This is accomplished by testing (i.e. exposing) a whole unmodified sample (which is then serially diluted) to a selection of organisms and measuring the toxic effect the sample has on each the bioassays endpoint (i.e. survival, growth, development, etc.). The toxicity data is then integrated using a SSD to derive species protection dilution factors for 99, 95, 90, and 80% species protection.

The SSD methodology outlined Warne et al. (2018) is designed for use with only a single contaminant (e.g. copper, zinc, phenol, etc.) and is the method for all of the ANZG (2018) default guideline values (DGVs) for toxicants. There is no specific guidance on conducting SSDs with DTA or samples that have a multi-toxicants or multi-stressors. In the absence of specific guidelines and methodologies for DTA the Warne et al. (2018) method has been adopted in Australia for DTAs. Thus, this bitterns DTA followed the methods and recommendations described therein to derive a high reliability guideline value including:

- A minimum of 8 species from at least 4 taxonomic groups
- The use of chronic bioassay over acute bioassays
- The derivation of EC_{10}s for each bioassay for use in the SSD

Further selection criteria for this assessment included the selection of species that best represent local species found in the coastal marine environment of the Pilbara region in the Northwest Western Australia.

MARINE ECOTOXICITY TESTING IN AUSTRALIA

In Australia there are currently only two commercial ecotoxicology laboratories that conduct DTA testing, Intertek (Geotechnical Services Pty Ltd) in Perth and Ecotox Services Australia Pty Ltd (ESA) in Sydney.

There are other government laboratories such as AIMS and CSIRO that have done commercial ecotoxicity testing in the past, but they either no longer do any commercial testing (CSIRO) or they are very limited on the types of commercial projects that they undertake (AIMS). There are Australian Universities that are active in ecotoxicity research; however, none are offering commercial ecotoxicology testing services to the authors knowledge.

Both commercial laboratories (Intertek and ESA) operate under NATA accreditation (ISO 17025) to ensure a high level of data quality with robust QA/QC practices to meet the requirements of regulators. Both labs offer a diverse selection of species that can be combined into test suites tailored to the specific DTA being undertaken.

STANDARD VERSUS SITE-SPECIFIC DIRECT TOXICITY ASSESSMENT

When conducting a DTA the selection of species to be included is a critical step. Species should represent a range of taxonomic groups, include a variety of biological endpoints (i.e. growth, germination fertilisation development, etc.), be sensitive to the physical or chemical stressors present in the discharge, and with the species ideally being found in the receiving environment. Unfortunately, it is not always possible to be able to meet all of these criteria. This often results in balancing standard versus site-specific ecotoxicity tests for the DTA. Both approaches have their pros and cons and this section will briefly cover these. For a more detailed discussion of this please see the ANZG (2018) guidance document titled *Guidance on the use of ecosystem receptor indicators for the assessment of water and sediment quality* (ANZG 2023).

Standard Ecotoxicity Test Methods

Standard toxicity tests have their advantages in that they use defined standardised methods which enables repeatability of test results as well as limiting confounding variables. This allows results from tests to be compared both over time for the same effluent as well as comparison to other effluents. The species used are often selected for their sensitivity to a wide range of contaminants and their sensitivity to model compounds is often already known. In addition, these standardised tests are often tested within a strong quality assurance and quality control framework to ensure data quality and repeatability.

Though using standard test methods for DTAs results in strong reproducible results, this approach can be limiting in that it only looks at very specific species which don't necessarily represent the receiving environment. Sensitive species are often selected, and exposure regimes can be seen as conservative (i.e. longer than would likely happen in situ), which results in standard DTA methods being more likely to overprotect aquatic ecosystems (Chapman 2000, Chapman et al. 1998).

Site Specific Ecotoxicity Test Methods

Site specific DTAs have the advantage that they are designed specifically for the relevant local conditions with species from the receiving environment, which can provide the best results for environmental protection. This is especially true when standard test species are found in the local environment or are at least considered a representative species of the local environment, but this is not always the case. When there are no standard test species available then new methods need to be validated. The development of new site-specific toxicity test methods is very costly and requires significant investment of time and resources often with no guarantee that a successful bioassay can be validated. In some cases, local species can be used with a standard method which can make validation more achievable.

A brief overview of test validation is given below as an example of the challenges faced in developing new bioassays. First bioassay species will have to be identified as a suitable bioassay species (i.e. considering the species life history, morphology, ecology and distribution). Secondly, the species will need to be sourced and they will need to either be able to be laboratory (or hatchery) cultured or field collected from a control site. If field collected is the only option, then transportation of individuals needs to be considered especially if the site is remote. Then a bioassay endpoint needs to be identified based on the lifecycle of the species and control experiments conducted to assess endpoint viability. Finally, the bioassay needs to be validated with model toxicants to define responses to toxicants.

Additional limitations of site-specific DTA test methods are that they are often one-off tests and therefore do not have the same QA/QC and validation as a standard test, especially compared to standard methods which have undergone NATA (ISO 17025) accreditation. Other considerations for site-specific DTA testing are that there is potential for confounding results due to background toxicity if local waters are used (Pifher and Egan 1989), the natural variability around bioassay endpoints may be unknown, limited information about the sensitivity to different contaminant classes, the ability to source individuals regularly may be limited (especially if there are seasonal constraints to spawning etc.), and results are not suitable for comparisons to other effluents (van Dam et al. 2019, ANZG 2023).

Balancing Standard and Site Specific Ecotoxicity Test Methods

In Australia the ANZG (2018) water quality guidelines have a tailored, not a prescriptive approach, with a focus on site-specific considerations and assessment criteria for water quality. This is also applicable to DTA testing. For example, there is no set standard DTA test suite like in the USA, instead the majority of DTAs select from the available standard bioassays to tailor the test suite to the location of the discharge as much as reasonably possible. This results in the majority of DTAs having at least a subset of species used that are considered local or regionally relevant and the other species selected are then prioritised on either the relationship to an important species / taxonomic group or due to their sensitivity to the type of discharge (e.g. salinity sensitive species will be chosen over salinity tolerant species for a brine discharge). This approach results in a balance between site-specific and standard testing, allowing it to have robust and repeatable results, while ensure that the local environment is accounted for.

While new bioassays continue to be developed, it takes time for the new test methods to be offered commercially and the development of new methods is often weighed against the demands for these tests (van Dam et al. 2019). This is especially true considering that Australia has a wide range of marine and coastal environments with a large diversity of marine life associated with each ecosystem, making it difficult to have the minimum of 8 bioassays for SSD available for all the different Australian marine ecosystems.

SELECTION OF TEST SPECIES AND BIOASSAYS

Leichhardt Salt requested Intertek to put together an appropriate ecotoxicity testing suite to undertake a DTA of a pre-production bitterns sample which was considered representative of the expected bitterns produced in their proposed solar salt field.

Intertek considered all commercially available test species currently used in Australia, including those offered at Intertek's Perth laboratory as well those offered by other labs such a Ecotox Services Australia. The bioassay selection process is detailed below.

The primary selection criteria were based on the ability to derive high reliability guideline values and dilution factors for the bitterns sample using the SSD methodology outlined by Warne et al. (2018). It is noted that the Warne et al. (2018) methodology is designed for single contaminants only and not for DTAs and although there is no specific guidance for conducting SSDs on DTAs in ANZG (2018), the data requirements are similar (ANZG 2023) and the Warne et al. (2018) methodology is commonly applied.

These requirements include:

- A minimum of 8 species from a minimum of 4 taxonomic groups
- Preference for chronic endpoints (as defined in Warne et al. 2018) over acute endpoints.
- All bioassays have a reliable calculated EC_{10} for use in the SSD
- Preference for species relevant to the Pilbara region in the NW of WA.

The focus was to attain the best DTA test suite currently available with representative species that are either local to the Pilbara region or are the best possible surrogates when a local species was not available. The selection process for each bioassay and test species has been broken down based on taxonomic group and is discussed in detail in the sections below. The final selected DTA test suite is detailed in Table 1.

Table 1: Bioassay Details

BIOASSAY	PROTOCOL	REFERENCE	TEST SPECIES	TEMP.
15-min Acute Microtox Bioassay	WIECX-17	Microbics 1992	*Vibrio fischeri*	15°C
72-hour Microalgal Growth Bioassay	WIECX-06	Stauber et al. 1994	*Isochrysis galbana*	22°C
72-hour Macroalgal Zoospore Germination Bioassay	WIECX-08	Burridge et al. 1999	*Ecklonia radiata*	22°C
48-hr Mollusc Larval Development Bioassay[2]	ESA Protocol	APHA 1998	*Saccostrea echinata*	29°C
72-hr Sea Urchin Larval Development Bioassay	WIECX-25	ASTM E1563a	*Echinometra mathaei*	25°C
1-hr Sea Urchin Fertilisation Bioassay [2]	ESA Protocol	USEPA 2002	*Heliocidaris tuberculata*	20°C
5-7 day Copepod Early Life Stage Bioassay [1]	WIECX-26	ISO 16778	*Gladioferens imparipes*	22°C

BIOASSAY	PROTOCOL	REFERENCE	TEST SPECIES	TEMP.
48-hr Copepod Immobilisation Bioassay [1]	WIECX-28	Gissi et al. 2013	*Acartia sinjiensis*	30°C
8-day Sea Anemone pedal lacerate development [2]	ESA Protocol	Howe et al. 2014	*Aiptasia pulchella*	25°C
7-day Fish Larval Development Bioassay	WIECX-16	USEPA 1005.0	*Seriola lalandi*	22°C

Note [1]: Non-NATA Accredited Method
Note [2]: Subcontracted test to Ecotox Services Australia

Bacteria

The Microtox bioassay was selected as a screening bioassay to help with setting the concentration series to be tested with the other bioassays. Please note that Microtox is not used in the SSD as it is not considered an environmentally relevant bioassay (Batley et al. 2018).

Microalgae

Microalgae are an important taxonomic group as they are primary producers and the base of many marine food webs. There are two species of micro algae commonly used in DTA testing *Isocrhysis galbana* (CS-177 now known as *Tisochrysis lutea*) and *Nitzschia closterium* (CS-114 now known as *Cylindrotheca closterium*) (please note that the older species names have been retained for consistency in nomenclature with previous testing).

Generally speaking, microalgae species are quite tolerant to higher saline brine and bitterns samples due to the additional micronutrients often present (Stringer pers. obs.). Therefore, only one microalgae sample was selected for this assessment. Despite both species being commonly used in DTA testing, *I. galbana* was selected over *N. closterium* as it is a tropical free-swimming flagellated microalgae species where *N. closterium* is a benthic diatom and that is more common in temperate waters though a there is a tropical strain commonly used in DTA testing.

Macroalgae

Macroalgae, like microalgae, are important primary producers in coastal ecosystems, with wide range of diversity. Additionally, macroalgae play an important role in ecosystem structure, providing habitat for many marine organisms. Currently in Australia there are only two species of macroalgae available for DTA testing the brown macroalgae species, *Ecklonia radiata* and *Hormosira banksii*. Both species are most commonly found in temperate Australian waters. There is currently no ecotoxicity bioassays developed for Australian tropical macroalgae species (van Dam et al 2008). Of the two species *E. radiata* has the more northern distribution including subtropical waters from Kalbarri and the Abrolhos Is, WA and across southern Australia and Tasmania up to Caloundra, Qld. (Wernberg et al. 2019). *H. banksii* has a more limited distribution being found primarily in southeastern Australia. Both species share the same ecotoxicity methodology, a 72-hr zoospore germination bioassay, utilizing the haploid zoospores released from the macroalgae. While there is a lack of tropical macroalgae species currently available for DTA testing, Burridge et al. (1995) suggested as brown macroalgae share similar reproductive strategies (i.e. via zoospore release), southern temperate species could be used as a surrogate for northern tropical ecosystems.

Leichhardt Salt requested testing with a *Sargassum* species. However, as there are currently no ecotoxicology tests available for *Sargassum* an alternate species was required. *Ecklonia radiata* was determined to be the most suitable surrogate species. This is firstly due to *Sargassum* being brown macroalgae species and using the opinion of Burridge et al. (1995) suitable as a surrogate species for tropical brown algae species. Secondly, of the two species available in Australia *E. radiata* has the most northern distribution being found in subtropical waters of WA and therefore considered a more representative species.

Molluscs

Currently there are five species of mollusc currently being used in ecotoxicity testing in Australia the blue mussel *Mytilus edulis,* the rock oyster *Saccostrea glomerata*, the milky oyster *Saccostrea echinata* (potentially being reclassified as *S. scyphophilla,* Krassoi pers. comm.), the pacific oyster *Crassostrea gigas* and the doughboy scallop *Mimachlamys asperrima*.

Of these only the milky oyster (*S. echinate*) is a tropical species and has a distribution from NT to southern Qld, though if it is reclassified as *S. scyphophilla* then it has a distribution from Geraldton WA to the NT (Snow et al. 2023). The distribution and habitat of the milky oyster make it an ideal species for the location of the proposed bitterns discharge.

Echinoderm

Echinoderms are an important and diverse group of marine organisms. Sea urchins are commonly used in ecotoxicity testing in Australia with two species being used, the temperate red sea urchin *Heliocidaris tuberculata* and the tropical rock-boring urchin *Echinometra mathaei*. Both species have two different ecotoxicology methods validated the 1-Hr Fertilisation Bioassay and the 72-hr Larval Development Bioassay and both methods are considered chronic tests. Both methods are sensitive to different contaminants and thus complement each other in a DTA assessment. *E. mathaei* is the most suitable test species for the bitterns assessment as it is local to the receiving environment. However, Warne et al. (2018) prohibits multiple data points from the same species being used in SSDs and though there is currently no guidance for conducting SSDs for DTAs in Australia, the guidance in Warne et al. (2018) was followed for this assessment. This resulted in both species being selected in this assessment. With the 1-Hr Fertilisation bioassay being conducted with *H. tuberculata* and the 72-hr larval development bioassay conducted with *E. mathaei.*

Crustacean

A wide range of marine crustaceans have been used in ecotoxicity testing worldwide, in Australia the common crustacean species include microcrustaceans such as copepods, amphipods and macrocrustaceans such as prawns and crabs (van Dam et al. 2008). Most microcrustaceans used in toxicity testing are cultured with in the laboratory providing a reliable source of naïve individuals that have not been exposed to contaminants previously. Macrocrustceans however are difficult to culture in the laboratory and thus the supply of individuals is based through commercial aquaculture facilities (van Dam et al. 2008). This makes ecotoxicity testing with macrocrustaceans more difficult as the supply is dependent on third parties, they can be seasonally dependant, and the transportation of some species (i.e. tiger prawns) can be restricted due to biosecurity laws (i.e. in WA they require a translocation permit).

For crustaceans there is a good review of Australian crustacean species that have been used in ecotoxicity testing in van Dam et al. (2008), but for the purpose of this project only available test species were considered. There are several species of amphipods that are commonly used in ecotoxicity testing but as they are a benthic species they are mainly used in sediment ecotoxicity testing. There are however some species used in aquatic water column exposures including *Melita plumulosa*, *Allorchestes compressa* and

Corophium sp. These amphipod species are temperate and not found in tropical waters. Additionally, the main methodology used for amphipods in DTA assessments is a 96-hr acute survival bioassay, as chronic amphipod tests have exposure of >6 weeks (van Dam et al. 2008) and are thus too long in duration for practical use. Therefore, amphipods were deemed not suitable for this assessment.

Copepods have proven to be a much more suitable group of species for ecotoxicity testing. There are three main species used for toxicity testing in Australia, *Acartia sinjiensis* which is common throughout tropical Australia (McKinnon & Duggan 2014), *Gladioferens imparipes* a WA endemic temperate – subtropical species and *Parvocalanus crassirostris* a euryhaline coastal species which is found in tropical and subtropical waters (McKinnon & Duggan 2014).

All three species are euryhaline species being able to tolerate a wide range of salinities (McKinnon & Duggan 2014, Payne & Rippingale 2001, Gissi et al. 2013, Binet et al. 2019). However, based on experience (Stringer pers. obs.) *G. imparipes* and *A. sinjiensis* are both more tolerant to lower salinities than higher salinities.

Both *G. imparipes* and *A. sinjiensis* are cultured in Intertek's Perth laboratory and regularly used for ecotoxicity and DTA assessments. Of the two species only *G. imparipes* currently has a commercially available chronic test method (a 5-7 day Larval development bioassay). A chronic methodology has been developed for *A. sinjiensis* by CSIRO (Binet et al. 2019) and Intertek is currently undergoing in-house validation of the chronic bioassay after *A. sinjiensis* cultures were made available to Intertek in 2023. Intertek has already completed the in-house validation of the 48-Hr sublethal immobilisation bioassay based on Rose et al. (2006) and Gissi et al. (2013). This acute bioassay is currently commercially available and the chronic is expected to be commercially available late 2024 - early 2025. *P. crassirostris* is currently used by Ecotox Services Australia using a 48-hr acute survival methodology, similar to that used for *A. sinjiensis* by Rose et al. (2006).

Leichhardt salt requested the inclusion of a prawn species but given the constraints of tiger prawns testing (i.e. juveniles only available seasonally and the biosecurity requirements) plus the 96-hr acute prawn survival test is known to be very tolerant to compared to other ecotoxicity tests (Stringer pers. obs.). In fact, these limitations were one of the primary driving factors for the development of the acute *A. sinjiensis* bioassay by Rose et al. (2006).

Therefore, this assessment chose the chronic 5-7 day larval development bioassay with *G. imparipes* and the 48-hr sub-lethal acute immobilisation bioassay with *A. sinjiensis.* Once the *A. sinjiensis* chronic bioassay is available, then there is an option for the sample to be re-tested to replace the acute data point in the SSD.

Sea anemone

Currently there is only one sea anemone bioassay validated in Australia, the 8-day Sea Anemone pedal lacerate development bioassay with the tropical sea anemone *Aiptasia pulchella.* As *A. pulchella* is a tropical species that has a distribution through the Indo- Pacific region it was thus deemed a suitable surrogate to local sea anemones species.

Fish

Fish are an important taxon to include into toxicity testing due to both their environmental value but also their commercial and recreational value. They are also the principal vertebrate species used in toxicity testing. There are currently only two species of fish able for commercial ecotoxicity testing in Australia the yellowtail kingfish (*Seriola lalandi*), and barramundi (*Lates calcarifer)*. Previously pink snapper (*Pagrus auratus*) and black bream (*Acanthopagrus butcheri*) were able to be used in DTA/ecotoxicity testing.

However, since the WA Department of Primary Industries and Regional Development's (DPIRD) hatchery in Fremantle, WA (previously known as Australian Centre for Applied Aquaculture Research) stopped maintaining their brood stocks of pink snapper and black bream (to focus on yellowtail kingfish) there has not been any brood stock available to supply the <24hr old eggs required for the toxicity testing. This leaves only barramundi and yellowtail kingfish as options for DTA testing.

Barramundi is a tropical species, with a distribution from Broome, WA to Brisbane, QLD. They are euryhaline species with adults primarily living in fresh to brackish waters and spawning in estuarine tidal flats (Grubert et al. 2020). There are two toxicity tests for barramundi the 96-hr imbalance test (sublethal) and the 7-day growth test (chronic) (ESA 2017).

Yellowtail kingfish are a pelagic species of fish that occupy temperate to sub-tropical waters with a distribution from Shark Bay, WA around southern Australia to Rockhampton, QLD (Huges et al. 2020). Intertek conducts a 7-day fish larval development bioassay with yellowtail kingfish which starts with <24hr old and encompasses egg development, hatching, and larval development through the yolk-sac larval stage to the flexion larval stage. This test covers the most sensitive development stages in fish larval development.

Leichhardt Salt had requested that local species to the Pilbara region be used for the testing such as blue spotted emperor, mangrove jack, gold spotted or black spotted cods. Unfortunately, there are no aquaculture facilities that have the brood stock of these species required for the toxicity testing with these species. Additionally, field collecting fish for toxicity testing is not practical for several reasons. If a chronic test similar to the 7-d larval development bioassay was to be considered, pre-spawning adults would have to be collected and artificially spawned prior to testing. This would be costly, time consuming, and require significant approvals from DPIRD. Similarly, if a juvenile life stage was used in an imbalance or growth bioassay the collection and proper identification of juvenile fish would be difficult and also require significant resources. It is the authors opinion that wild collecting of fish species is not a viable option for ecotoxicity testing.

Other tropical fish species that have been previously used or proposed as potential species are reef fish commonly found in the aquarium trade, such as the clown fish (e.g. *Amphiprion clarkia*), angelfish, or damsel fish species. Though due to the limited habitat (i.e. reefs) of these species they have been considered not appropriate for the majority of tropical marine DTAs.

Given the limitations of available methods for DTA testing with fish, the 7-day fish larval development bioassay with yellowtail kingfish was chosen over the barramundi bioassays as the most suited species for this discharge. This is primarily due to two factors, the first being yellowtail kingfish is a coastal to pelagic species, while barramundi is a euryhaline estuarine species with wider salinity tolerances. Secondly the 7-day fish larval development bioassay is a true chronic bioassay (as defined in Warne et al. 2018) covering the most sensitive life history stages and thus provides the best data as a surrogate for the local species of the Pilbara region.

Intertek is committed to assisting in the development of more tropical fish ecotoxicity bioassay. However, due to the limited species available in aquaculture facilities it has proved difficult to develop tests for other species tests. In the case where brood stock of another species is identified and available, Intertek is willing to develop new bioassays for the species.

CONCLUSIONS

The selected bioassay species detailed above encompass 7 trophic groups (8 including Microtox) with a total of 9 species used for the evaluation of the ecotoxicity of the bitterns sample and calculation of the species protection dilution factors. The species represent key species group that are found in the receiving environment. While a completely site specific DTA was not completed, the best available local species were included. Where local species were not available, the most suitable surrogate species were used, either a related species or a species that is a sensitive indicator species. In addition, a variety of biological endpoints were used including growth, larval development, immobilisation, fertilisation, and germination.

As a wide range of relevant species and biological endpoints were included and the use of standard species in DTA assessment has been shown to provide more conservative environmental protection (i.e. overprotect) (Chapman 2000, Chapman et al. 1998), the proposed test suite should provide a high level of environmental safety for this proposed bitterns sample. In addition, as the species proposed are commercially available and not one-off tests, future assessments for this salt field bitterns will be able to repeat this DTA test suite which will allow for comparison of results from pre-commissioning, commissioning, as well as routine discharge monitoring over the lifecycle of the project.

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APPENDIX

SUPPLEMENTARY INFORMATION

Bioassay's Identified and Evaluated for Application to DTA for ESSP Proposal

Count	Taxonomic Group	Species	Details	Acute/ Chronic	Yes or No Parameters					Summary from technical
					Currently Available	NATA Accredited	Tropical Australia Species	Likely Local to Proposal	Applied to ESSP DTAs	
	Bacteria	*Vibrio fischeri*	15-min bioluminescence	na	Y	Y	N	N	Y	Microtox
1	Crustacean	*Corophium sp.*	96-h survival	acute	Y	N	N	N	N	acute test, not found in tropical Australia
2		*Allorchestes compressa*	96-h survival	acute	Y	N	N	N	N	acute test, not found in tropical Australia
3		*Melita plumulosa*	96-h survival	acute	Y	N	N	N	N	acute test, not found in tropical Australia, NSW estuarine environment.
4		*Acartia sinjiensis*	48-h immobilisation	acute	Y	N	Y	Y	Y	common throughout tropical Australia, estuarine more tolerant to lower salinities.
5			80-h larval development ratio	chronic	N	N			N	Chronic test is under development, but not yet proven
6		*Gladioferens imparipes*	7-d larval development ratio	chronic	Y	N	Y	N	Y	WA endemic temperate - subtropical
7		*Parvocalanus crassirostris*	78-h survival	acute	Y	N	Y	Y	N	acute test
8	Echinoderm	*Echninometra mathaei*	72-hr larval development	chronic	Y	Y	Y	Y	Y	most suitable as it is local to the receiving environment, commonly used for Ecotox testing in Australia
9			1-hr fertilisation	chronic	Y	Y			N	
10		*Heliocidaris tuberculata*	1-hr fertilisation	chronic	Y	Y	N	N	Y	temperate red sea urchin, commonly used for Ecotox testing in Australia
11			72-hr larval development	chronic	Y	Y			N	
12	Fish	*Lates calcarifer*	7-d growth	chronic	Y	N	Y	N	N	tropical species distribution from Broome to Brisbane, euryhaline species living in fresh to brackish water, spanning in estuarine tidal flats, high salinity tolerant
13			96-hr fish imbalance	acute	Y	N			N	
14		*Seriola lalandi*	7-d larval development	chronic	Y	Y	N	N	Y	pelagic, temperate to sub-tropical waters-Shark Bay to Rockhampton
15		*Pagrus auratus*	7-d larval development	chronic	N	Y	Y	Y	N	brood stock no longer available
16		*Acanthopagrus butcheri*	7-day larval development	chronic	N	Y	N	N	N	brood stock no longer available, Estuarine fish
17	Macroalgae	*Ecklonia radiata*	72-hr zoospore germination	chronic	Y	Y	N	N	Y	has the more northern distribution, inc subtropical Kalbarri & Abrolhos
18		*Hormosira banksii*	72-hr zoospore germination	chronic	Y	N	N	N	N	limited distribution, primary southeastern Australia
19	Microalgae	*Nitzschia closterium* (now *Cylindrotheca closterium)*	72-hr growth	chronic	Y	Y	Y/N	N	N	benthic diatom, two strains used in ecotox testing one temperate (CS- 1) and one tropical (CS-114)
20		*Isochrysis galbana* (now *Tisochrysis lutea)*	72-hr growth	chronic	Y	Y	Y	Y	Y	tropical free-swimming flagellated microalgae
21	Mollusc	*Mytilis edulis*	48-hr larval development	chronic	Y	Y	N	N	N	
22		*Saccostrea glomerata*	48-hr larval development	chronic	Y	Y	N	N	N	
23		*Crassostrea gigas*	48-hr larval development	chronic	Y	Y	N	N	N	
24		*Mimachlamys asperrima*	48-hr larval development	chronic	Y	Y	N	N	N	
25		*Saccostrea echinata*	48-hr larval development	chronic	Y	Y	Y	MAYBE	Y	tropical species, NT to southern Qld, if it is reclassified as *S. scyphophilla* then it has a distribution from Geraldton WA to the NT
26	Cnidarian	*Aiptasia pulchella*	96-h survival	acute	Y	N	Y	N	N	only sea anemone bioassay validated in Australia, tropical species distribution through IndoPacific region
27			8-d pedal lacerate development	chronic	Y	N			Y	
			Notes:							
			red refer to undesirable characteristics							
			Available taxonomic groups		7	5	6	2	7	
			Available test species		20	11	7	3	9	
			Available bioassays		24	15	11	4	9	