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Sensitivity Assessment of Contaminant Pressures – Selected Polychaetes – Evidence review

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1 Introduction

An evidence review of the effects of contaminants on selected species of polychaetes was undertaken between December 2022 and March 2023. The evidence review followed the Rapid Evidence Assessment (REA) protocol developed previously (Tyler-Walters *et al.*, 2022).

The resultant 'Polychaetes Evidence Summary' spreadsheet (available here) and 'evidence review' that follows benefited from improvements and resultant minor adjustments. The 'evidence summary' template was updated to improve data entry. The improvements included:

- the addition of both the reported and standardised values for the exposure concentrations of contaminants used (where available);
- the addition of both the reported and standardised values for the observed or effect concentrations of contaminants (where available);
- use of 'common' or 'short' names for chemicals derived from the PubChem[1] database where possible, and
- a standard 'summary narrative' writing style was adopted for consistency in reporting.

In addition, 'contaminant type' is recorded as the function of the chemical (e.g., herbicide, analgesic), rather than the structure of the chemical (e.g., organohalogen, organophosphate), if the information allows.

All the technical terms used in the 'Anthozoa Evidence Summary' and the report that follows are defined in Appendix 1.

[1] https://pubchem.ncbi.nlm.nih.gov/

2 Evidence review overview

The literature review focused on *Ampharete* spp., *Aphelochaeta* spp., *Arenicola* spp., *Hediste* spp., *Nereis* spp., *Nephtys* spp., *Pygospio* spp., *Eteone* spp., *Lanice* spp., *Streblospio shrubsolii* and the oligochaete *Tubificoides* spp.. Other polychaete genera were excluded to keep the literature review manageable. Please note, while *Hediste* is the accepted genus for many species previously described as *Nereis* (e.g., *Hediste* (syn. *Nereis*) *diversicolor*), *Nereis* remains an accepted genus. Therefore, both genera are used throughout, and the original name reported in each article/study is used in the evidence review and summary spreadsheet.

The initial searches (12th December 2022) resulted in ca 2,913 hits of which 1,645 were duplicates (Table 2.1) using the standard search strings developed previously (Tyler-Walters *et al.*, 2022). Only the Web of Science (WoS) science citation index and the ECOTOX[2] Knowledgebase were used due to time constraints. The resultant references were screened for relevance based on the proposed REA protocol. Screening against the exclusion criteria reduced this number to 86 articles[3], which were taken forward for detailed review. However, 14 articles could not be accessed, even using inter-library loans. Only articles written in English or with readily available English translations were included.

Table 2.1. Results of literature review for 'polychaetes'.

Review stage	No. articles identified/retained	No. articles rejected/removed
Web of Science	2,734	
ECOTOX database	179	
Duplicates removed	1,089	1,645
Screening	101	988
Taken forward*	87	
Not accessible	9	

* Does not include further articles identified from the articles reviewed, or alternative sources

[2] https://cfpub.epa.gov/ecotox

[3] The term 'article(s)' or 'study' are used for peer reviewed papers, reports, and other publications relevant to the review.

Overall, the articles reviewed reported mortality (‘Severe’ to ‘Some’) in 50% of results (worst-case ranked mortalities), ‘No’ mortality (‘none’) in 10% of results, and sublethal effects in 36% of results. The level of mortality or sublethal effect was ‘unspecified’ in the remaining 4% of results. Most sublethal effects were reported in studies of the effects of ‘Transitional metals’ on polychaetes (Figure 2.1).

Figure 2.1. Count of worst-case ranked mortalities due to exposure to contaminants in selected polychaetes. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects

‘Transitional metals’ (henceforth ‘Metals’) was the most studied contaminant group and contributed 52.8% of the results in the evidence review. ‘Pharmaceuticals’ contributed 12.4%, 'Pesticides/biocides' 9.8%, petrochemical hydrocarbons contributed 8.5%, and the remaining groups of contaminants contributed less than 16.5% of the results (Figure 2.1). Due to the high proportion of sublethal effects reported for metals, only those articles that reported mortality as an effect are documented in the body of the report, although all the evidence collated is included in the 'Polychaetes Evidence Summary' spreadsheet.

Arenicola spp. and *Nereis* spp. were the most studied genera and each contributed ca 33% of the results in the evidence reviewed, closely followed *Hediste* spp., which contributed 28% of the results (Figure 2.2). The remaining genera each contributed less than 1.5% of the results in the evidence reviewed.

Lanice spp. and *Aphelochaeta* spp. were mentioned in only two studies (Conlan *et al.*, 2004; Bergayou *et al.*, 2019). In both cases, the studied reported the effects of organic/nutrient enrichment due to wastewater discharges from sewage treatment works, which are outside the scope of this study.

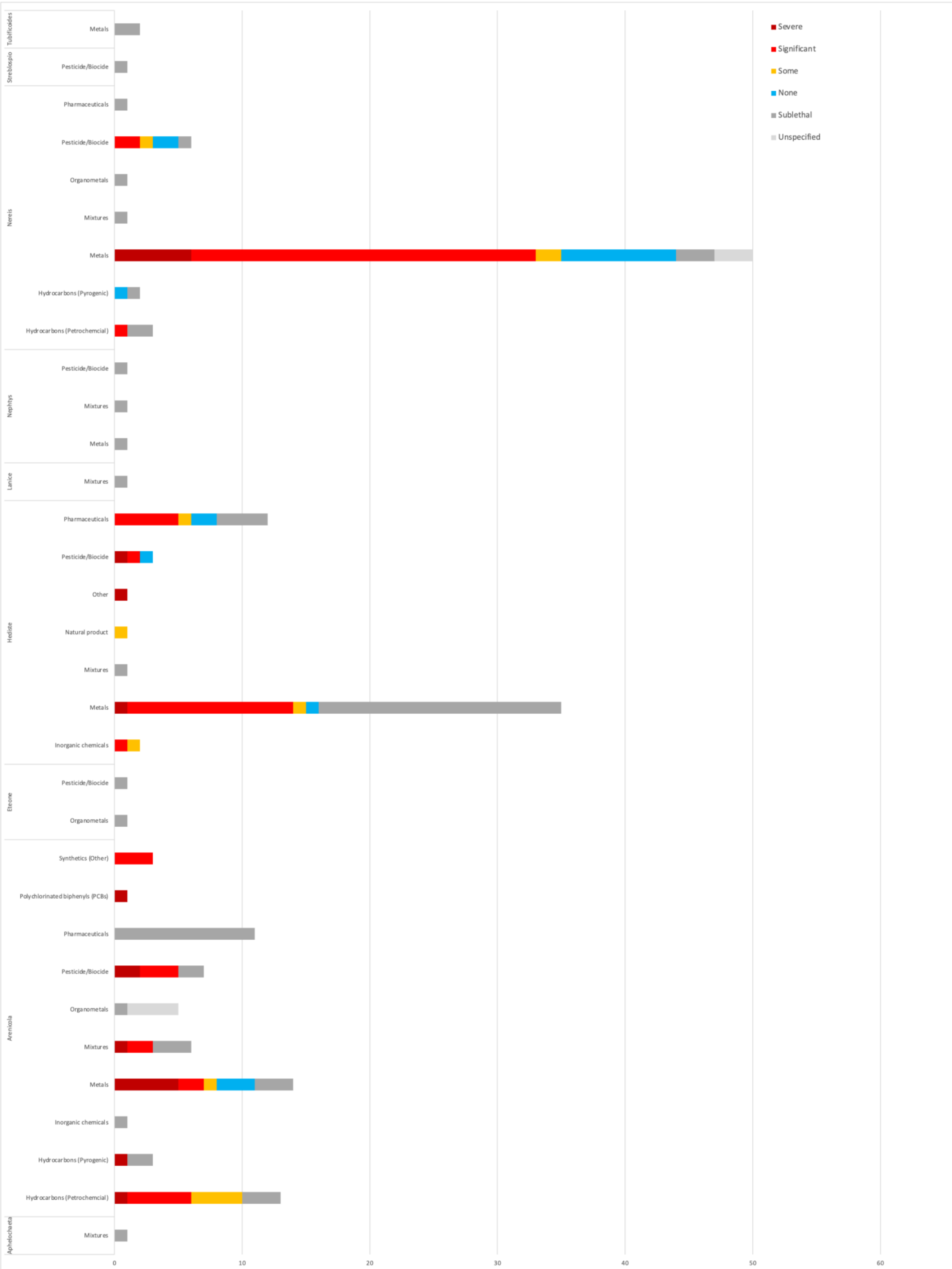

Figure 2.2. Count of worst-case ranked mortalities due to exposure to contaminants in selected polychaetes by genus. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

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3 Hydrocarbons and PAHs

At total of 23 results were obtained from seven articles that studied the effect of hydrocarbons, PAHs, and dispersants on polychaetes, of which four articles examined the effects of complex hydrocarbons such as crude or fuel oils and/or their water accommodated or saturated fractions (WAF/WSF).

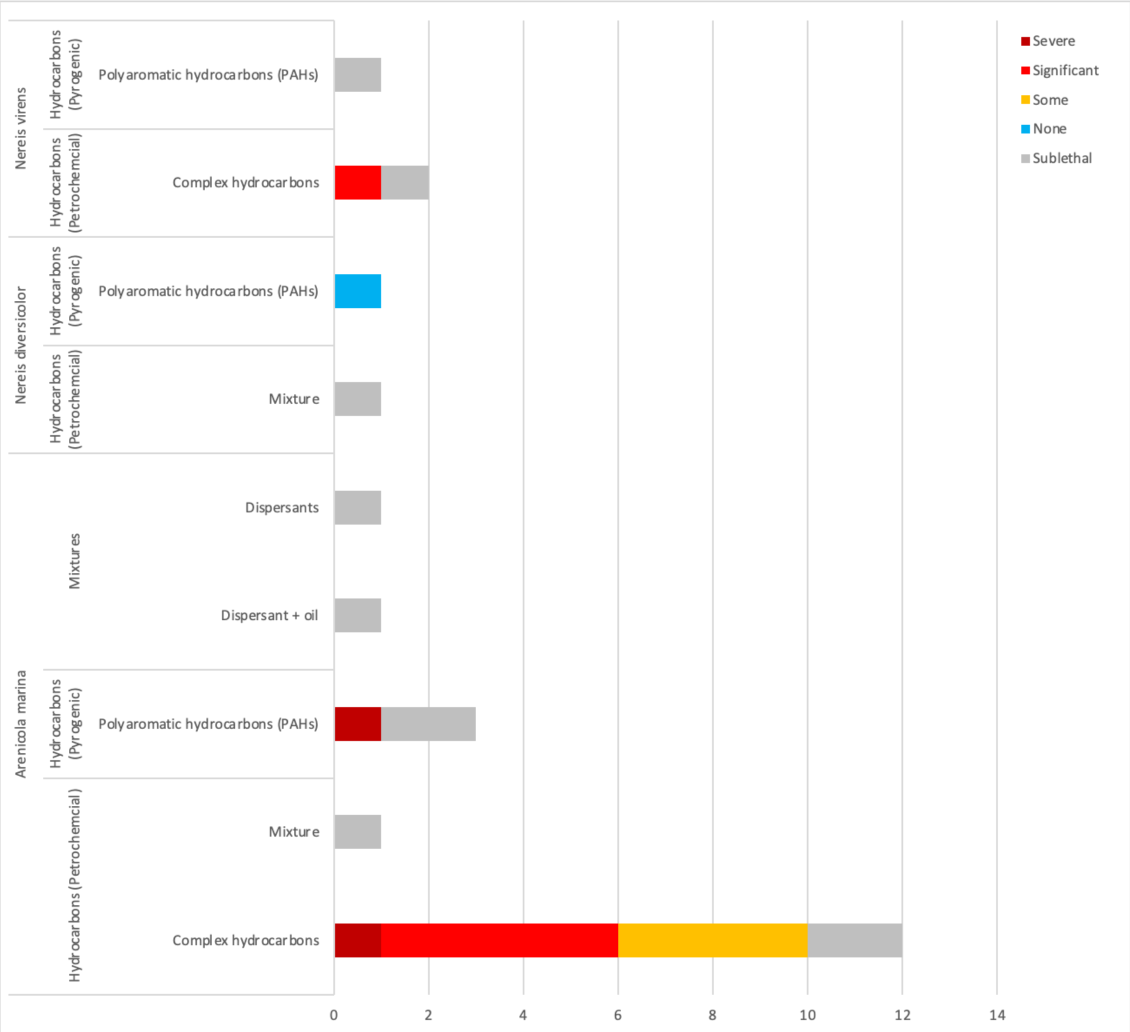

Figure 3.1. Count of worst-case ranked mortalities due to exposure to hydrocarbons in selected polychaetes species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

3.1 Petroleum hydrocarbons – oils and dispersed oils

Five articles examined the effects of petroleum oils (e.g., crude oil and fuel/bunker oils), and dispersed oils, and are summarized below.

Lewis *et al.* (2008) investigated the reproductive toxicity of water accommodated fractions (WAF) of crude oil on the polychaetes *Arenicola marina* and *Nereis virens*. In the fertilization success tests, fertilization occurred under exposure conditions during a 10-minute period, in which the gametes were exposed to 1, 10, and 100% WAF, as well as a positive control of 25µg/l fluoranthene. Oocytes were washed and left to develop for 24 hours after the 10-minute exposure period. Fertilization success was significantly reduced in both species by exposure to WAF and fluoranthene. Only 26.8% of oocytes showed signs of fertilization in *Arenicola marina* and 76% showed signs of fertilization in *Nereis virens* exposed to 100% WAF. No significant effects were observed on the embryos development 24-hour post-fertilization in *Arenicola marina*. However, the WAF exposures had an impact on the developmental rates of *A. marina*. After 24 hours post-fertilization, the fresh water and solvent control treatments had 24–43% of the surviving embryos at the swimming trochophore stage. However, no trochophores were present in the WAF treatments. Significant effects of WAF on post-fertilization development were observed in *N. virens*, with significant developmental abnormalities observed in the 10 and 100% WAF concentrations and in the fluoranthene treatment. The effects of WAF on early larval survivorship in *N. virens* larvae from fertilization in fresh seawater (development only in WAF) and fertilizations in WAF (fertilization and development in WAF) were significantly reduced in both experiments. Survival was more affected in the fertilization and development WAF treatments. For larvae fertilized in fresh seawater and exposed to WAF during early development, the survival was reduced to 55.6% in the 100% WAF treatment after 10 days. However, the larvae exposed to WAF during both fertilization and early development had 100% mortality in the 100% WAF treatment by day 5 and the survival in the 10 and 1% WAF treatments was reduced compared to the survival from the ‘development only’ experiment.

Morales-Caselles *et al.* (2008) investigated the toxicity and bioaccumulation associated with the contaminants present in the fuel oil extracted from a sunken tanker, using the lugworm *Arenicola marina*. Lugworms were exposed to sediment from the Bay of Cádiz (South of Spain) mixed with fuel oil extracted from the tanker at 0.5, 1, 2, 4, and 8% dry weight for 21 days. Lugworms were sampled to determine the mortality and the levels of individual PAHs in the organisms, after 10 and 21 days of exposure. Mortality was recorded daily and LC50 values for 10 and 21-day exposures were established at 6.4% and 2.4%, respectively. Mortality was positively related to dose and time of exposure. Complete (100%) mortality occurred in the 21-day 8% fuel oil treatment.

Morales-Caselles *et al.* (2009) used a multi-biomarker approach using the polychaete *Arenicola marina* to assess oil-contaminated sediments. Sediment from the Bay of Algeciras (south Spain) impacted by multiple, low-level contaminant inputs, and the Galician Coast (NW Spain), historically impacted by an oil spill (*Prestige* 2002) along with sediment from two reference sites selected in the UK were used. *Arenicola marina* was exposed to the marine sediments in the laboratory for 14 days. After 14 days of exposure, feeding and burrowing behaviour tests were carried out by moving the worms to clean sediment and recording the time it took for the worms to re-bury themselves and by removing casts and weighing casts to establish the feeding rates. Moderate to strong correlations between organics, metals, and biological responses were observed, with longer burrowing times and less casts produced.

McLusky & Martins (1998) investigated the long-term effects of petrochemical discharge on the faunal composition of an estuarine mudflat over 20-year period. The study location was the Kinneil intertidal area, in the middle reaches of the Forth estuary, eastern Scotland, which has been subject to the effects of industrial discharges, principally from petrochemical industries (oil refinery and chemical works) since the 1920s. The intertidal fauna in the estuary had been studied annually since 1976, providing over 20 years of data from 90 stations in the estuary. During the study period, the discharges into the estuary had been reduced through a combination of plant closure and the installation of effluent treatment works. In addition, the River Avon that flows across the area had experienced substantial improvements in water quality. *Nereis diversicolor* showed substantial changes in mean abundance over the study period, but no significant trends were recorded.

Farke & Gunther (1984) studied the effects of oil and dispersant on intertidal organisms in field experiments with a mesocosm over eleven months. Three field experiments on the effects of oil, a dispersant and an oil/dispersant mixture were carried out in an intertidal mud flat ecosystem. The fate of the oil in the sediment and the effects on phytobenthos, bacteria, and macrozoobenthos were studied. In the oil experiment, Arabian light crude oil was used at a concentration of about 2 mg/l. In the dispersant experiment, Finasol OSR 5 was used at a concentration of about 4 mg/l. In the dispersant/oil mixture experiment a combination of Arabian light crude oil and Finasol OSR 5 oil was used at a ratio of 10:1, with a hydrocarbon concentration of about 4 mg/l in the experimental caisson. In the oil exposure treatment, reductions in the feeding activities of the *Arenicola marina* were observed with reductions in the number of casts and a reduction in cast weight. In the oil/dispersant experiment, the number of casts was reduced by 25%, but the cast weights increased. The dispersant experiment showed no effects, on the number of casts or the weight of the casts.

3.2 Dispersants

The effects of dispersants alone were examined by one article, Farke & Gunther (1984), which is summarized above in section 3.1.

3.3 Polyaromatic hydrocarbons (PAHs)

The effects of polyaromatic hydrocarbons (PAHs) of pyrogenic origin were examined by three articles. The evidence is summarized below, except for Lewis *et al.* (2008) which is summarized above in section 3.1.

Casado-Martinez *et al.* (2008) investigated the suitability of lugworms (*Arenicola marina*) to study the bioaccumulation potential of mercury (Hg), PCB, and PAH compounds from dredged sediments in laboratory exposures. During the study, the lugworms were exposed to numerous sediment samples from several harbours that are important fishing and shipping ports near major centres of population, and, in areas mainly affected by historic mining activities but also hosting several industrial activities. The concentrations of Hg in sediments ranged between 0.05 and more than 136 mg/kg dry weight. A significant correlation between lugworm mortality and Hg concentration in sediments was observed up to 80%. However, as the control sediment showed 15–20% mortality there was the potential that mortality could have been caused by other sediment conditions and not related to chemical stress. PAH and PCB sediment concentrations were not correlated with mortality.

Catalano *et al.* (2012) investigated the suitability of *Hediste diversicolor* as a good candidate for evaluating PAH contamination. The study exposed ragworms to 100 and 500 µg/l benzo[a]pyrene (BaP) for a period of 10 days. Mortality was less than 10% and not influenced significantly by exposure.

3.4 Sensitivity assessment – Hydrocarbons and PAHs

The count of ranked mortalities due to 'Hydrocarbons and PAHs' are summarized in Figure 3.1 and in Table 3.1 below. The data presented in Table 3.1 include all life stages and articles where life stage were not reported or were unspecified (NR). Only seven articles examined the effects of petrochemical hydrocarbons, dispersant or their mixtures, and PAHs on the selected polychaetes reviewed. Furthermore, those studies only examined three of the selected genera, *Nereis*, *Hediste*, and *Arenicola*.

The resilience ranks used in the sensitivity assessments that follow are derived from the MarLIN sensitivity dataset (Tyler-Walters *et al.*, 2023; www.marlin.ac.uk) unless stated otherwise. For example, the resilience of *Nereis virens* is assumed to be similar to *Hediste* (syn. *Nereis*) *diversicolor*. MES (2010) suggested that the genus *Nereis* spp. had a high recoverability.

Table 3.1. Summary of count of 'worst-case' ranked mortalities to 'Hydrocarbons and PAH' contaminants reported in the evidence review and resultant proposed sensitivity assessments for polychaete species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Species Name	Group/Type	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Arenicola marina										
	Complex hydrocarbons	**1**	5	4		2	12	N	M	M
	Mixture[4]					**1**	1	H	H	NS
	Polyaromatic hydrocarbons (PAHs)	**1**				2	3	N	M	M
	Dispersant + oil					**1**	1	H	H	NS
	Dispersants					**1**	1	H	H	NS
Total		**2**	**5**	**4**		**7**	**18**	N	M	M
Nereis diversicolor										
	Mixture[5]					**1**	1	H	H	NS
	Polyaromatic hydrocarbons (PAHs)				**1**		1	H	H	NS
Total					**1**	**1**	**2**	**H**	**H**	**NS**
Nereis virens										
	Complex hydrocarbons		**1**			1	2	L	H	L
	Polyaromatic hydrocarbons (PAHs)					**1**	1	H	H	NS
Total			**1**			**2**	**3**	**L**	**H**	**L**
Overall total		**2**	**6**	**4**	**1**	**10**	**23**			

No species-specific information on oil spills was recovered in the literature review. However, Suchanek (1993) reviewed the effects of oil spills and concluded that soft sediment polychaetes, bivalves, and amphipods were particularly sensitive. Dauvin (2000) noted that

[4] Oil contaminated sediment
[5] Petrochemical discharges on an estuarine mudflat (McLusky & Martins, 1998)

20 years after the *Amoco Cadiz* oil spill in 1978, *Lanice conchilega* was re-established between 1978 and 1984 but disappeared after 1985, although the cause of its disappearance was uncertain. A similar delayed response was observed by Sanders (1980) and Kingston *et al*. (1995) as a result of the *Florida* oil spill and the *Braer* oil spill (Gómez Gesteira & Dauvin, 2000). The *Amoco Cadiz* oil spill resulted in reductions in abundance, biomass, and production of the affected invertebrate communities. However, *Nephtys hombergii* and other polychaetes (cirratulids and capitellids) were largely unaffected by the *Amoco Cadiz* oil spill (Conan, 1982). The sediment rapidly recovered and in 1981, benthic recruitment occurred under normal conditions (Dauvin, 1998). The evidence suggests that some polychaetes are largely unaffected, especially if opportunistic or mobile, and/or able to recover quickly after the initial spill.

3.4.1 *Arenicola* spp.

Lewis *et al.* (2008) reported that both the WAF of crude oil and the PAH fluoranthene adversely affected fertilization success and caused larval mortality in *A. marina*. Morales-Caselles *et al.* (2008) noted that sediment contaminated with fuel oil from a sunken tanker caused significant mortality in *Arenicola marina*, for example, 8% fuel oil / dry weight sediment resulted in 100% mortality after 21 days. Farke & Gunther (1984) and Morales-Caselles *et al.* (2009) demonstrated that oil-contaminated sediment reduced burrowing activity in *A. marina*. Farke & Gunther (1984) noted that dispersed oil also reduced burrowing activity but the dispersant used showed no effect. The evidence suggests that oil contamination could also reduce recruitment to contaminated sediment, and hence prolong recovery. However, exposure of adult *A. marina* to PAHs (Casado-Martinez *et al.*, 2008) did not contribute to mortality.

Levell (1976) examined the effects of experimental spills of crude oil and oil-dispersant (BP1100X) mixtures on *Arenicola marina*. Single spills caused 25-50% reduction in abundance and an additional reduction in feeding activity. Up to four repeated spillages (over a 10-month period) resulted in complete eradication of the affected population either due to death or migration out of the sediment. Levell (1976) noted that recolonization was inhibited but not prevented. Prouse & Gordon (1976) examined the effects of surface fuel oil contamination and fuel oil sediment mixtures on the blow lug in the laboratory. They found that the blow lug was driven out of the sediment by a waterborne concentration of >1 mg/l or sediment concentration of >100 µg/g. Worms forced out of sediment may be able to migrate out of the affected area but will be exposed to severe predation risk, especially in daylight.

Seawater oil concentrations of 0.7 mg oil/l reduced feeding after 5 hours and all worms exposed for 22 hours to 5 mg/l oil left the sediment and died after three days. However, the sample size in this experiment was very small (six worms). A sediment concentration >10 µg/g could reduce feeding activity. *Arenicola marina* can recolonize sediment relatively quickly (within one month), however, contaminated sediments would probably take longer to recover, extending recovery times.

Therefore**, the resistance of *Arenicola marina* to petrochemical hydrocarbons is assessed as 'None' based on the worst-case results reviewed. Hence, resilience is 'Medium' and sensitivity is assessed as 'Medium'.** The evidence suggests that dispersed oils and dispersants only caused sublethal effects. Hence, **its sensitivity to dispersed oils and dispersants is also assessed as 'Medium'** but with 'Low' confidence due to the limited number of studies reviewed. The PAH flouranthene was reported to cause severe mortality in *A. marina* larvae, which may result in population decline in the long-term. Therefore, **the resistance of *A. marina* to PAHs is assessed as 'None' based on the worst-case results reviewed but with 'Low' confidence. Hence, resilience is 'Medium' and sensitivity is assessed as 'Medium'.**

3.4.2 *Nereis* spp.

As above, Lewis *et al.* (2008) reported that both the WAF of crude oil and the PAH fluoranthene adversely affected fertilization success in *N. virens*, while the WAF caused 'Significant' larval mortality. No mortality due to PAH exposure was reported in the evidence reviewed. McLusky & Martins (1998) investigated the long-term effects of petrochemical discharge on the faunal composition of an estuarine mudflat over a 20-year period. The abundance of *N. diversicolor* varied over the 20 years and but no significant trends were recorded.

Therefore**, the resistance of *Nereis* spp. (and *Hediste* spp.) to petrochemical hydrocarbons is assessed as 'Low' based on the worst-case results reviewed. Hence, resilience is 'High' and sensitivity is assessed as 'Low'** on the assumption that fertilization failure could result in long-term population decline but with 'Low' confidence due to the limited evidence reviewed.

3.4.3 *Nephtys* spp. and *Eteone* spp.

Nephtys hombergii and other polychaetes were reported to be unaffected after the *Amoco Cadiz* spill while the *West Falmouth* spill eradicated the benthos. McLusky (1982) found that petrochemical effluents, including organic solvents and ammonium salts, released from a point source to an estuarine intertidal mudflat of the Forth Estuary, Scotland, caused severe pollution in the immediate vicinity. Beyond 500 m distance, the effluent contributed to an enrichment of the fauna in terms of abundance and biomass similar to that reported by Pearson & Rosenberg (1978) for organic pollution. *Nephtys hombergii* was found in low numbers in the area with a maximum abundance of species and the highest total biomass at 500 m from the discharge. Its abundance was greatest at 1.5-2 km from the discharge, while *Eteone* spp. and spionids were most abundant at 1-1.5 km (McLusky, 1982). However, the petrochemical discharge polluted the sediment within 500m of the discharge but beyond that the effects were due to organic enrichment rather than the toxicity of petrochemicals alone (McLusky, 1982).

Therefore, **the worst-case resistance of *Nephtys* spp. to petroleum hydrocarbons is probably 'Low'** based on the effects of the West Falmouth spill and petroleum discharge but with 'low' confidence due to the limited evidence. **Hence, resilience is assessed as 'High' and sensitivity as 'Low'. Similarly, the sensitivity of *Eteone* spp. is also probably 'Low'.**

4 Transitional metals and organometals

A total of 102 results (ranked ‘worst-case’ mortalities) were obtained from 55 articles that examined the effects of transitional metals and organometals on polychaete species (Figure 4.2). *Hediste* (*Nereis*) *diversicolor* was the most studied species, which provided the most results from studies of metals (69.6%). *Arenicola marina* was the next studied species with 13.7% of results for metals, then *Nereis virens* with 10.8%, followed by *Tubificoides* spp. and *Nephtys caeca* with less than 2% each (Figure 4.1). Only one of the screened articles assessed the effects of organometals on polychaetes (*Arenicola marina*, *Eteone* spp. and *Hediste* (*Nereis*) *diversicolor*).

4.1 Transitional metals

The most studied metals were in the order Copper (Cu) > Zinc (Zn) >Silver (Ag) >Cadmium (Cd) = Mercury (Hg) (Figure 4.2). Tributyltin was the only organometal examined in the articles reviewed. Most of the results (40%) on the effects of transitional metals did not report the life stage of the organism. However, it is assumed that these will either be adults or juveniles. Hence, 78.5% of the results on the effects of ‘metals’ examined adult/juvenile life stages, followed by gametes (12.5%) and early life stages (9%) (Figure 4.3). The evidence from the articles that reported mortality is summarized below.

Alla *et al.* (2006b) investigated the toxicity of copper and zinc on *Nereis diversicolor* individuals from a contaminated site and from a relatively clean site. For copper, at the two lowest tested metal concentrations (0.316 and 1.00 µM) no mortality occurred in either population. At the highest tested concentration of copper (8 µM), worms from the contaminated site had an LC50 of 25 hours, however, worms from the relatively clean site had 100% mortality. For zinc, at the two lowest tested metal concentrations (100 and 178 µM) no mortality occurred for worms from the contaminated site, however, LT50s of 93.11 and 48.42 h were established for worms from the clean site. At the highest tested concentrations of zinc (316.0, 562.0, 800.0 µM), LT50 were significantly higher for worms from the contaminated site than those from the clean site.

Figure 4.1. Count of ‘worst-case’ ranked mortalities due to exposure to metals, organometals, and nanoparticulate metals in selected polychaetes species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported/significant) and sublethal.

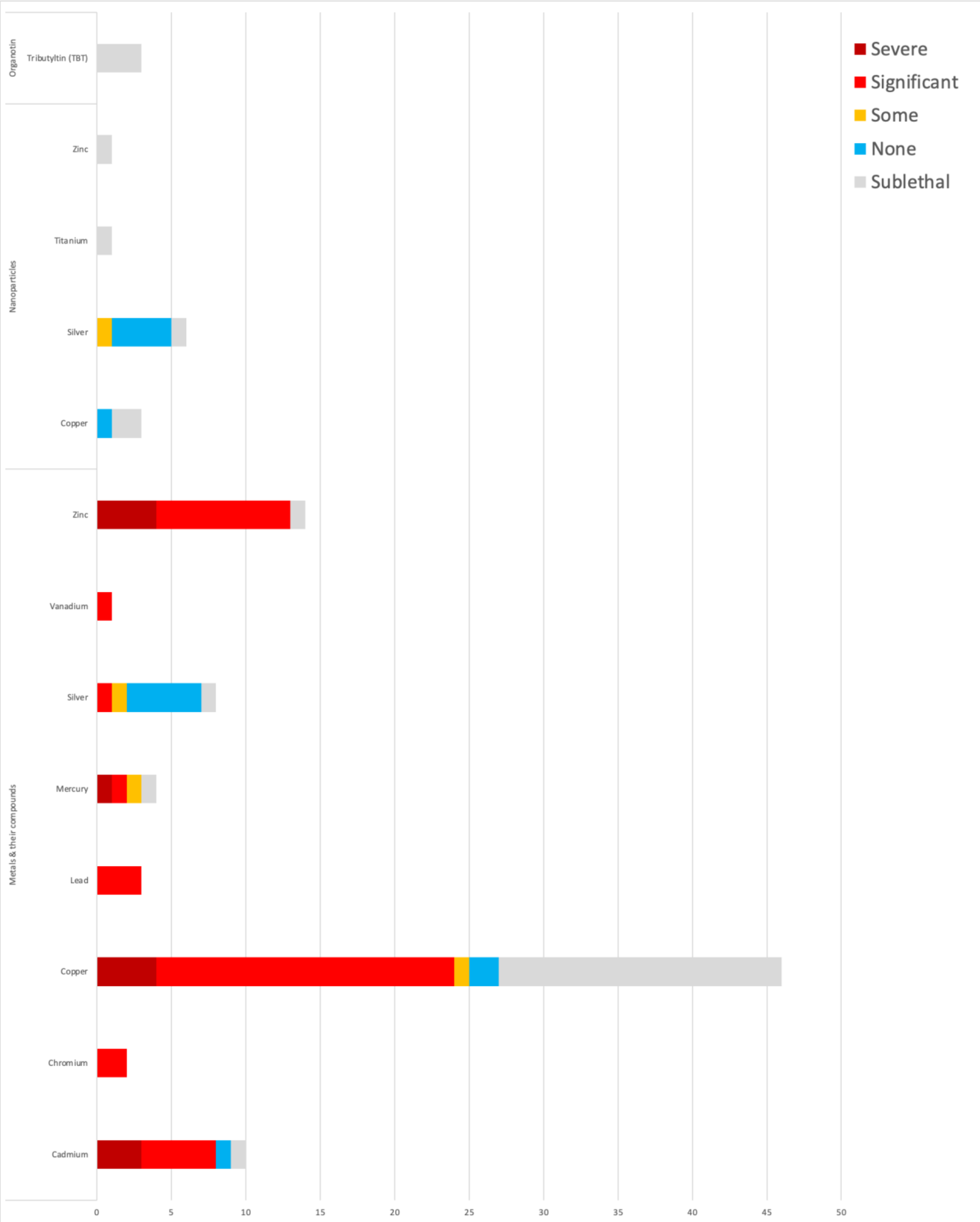

Figure 4.2. Count of ‘worst-case’ ranked mortalities due to exposure to metals, organometals and nanoparticulate metals in selected polychaetes. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported/significant) and sublethal.

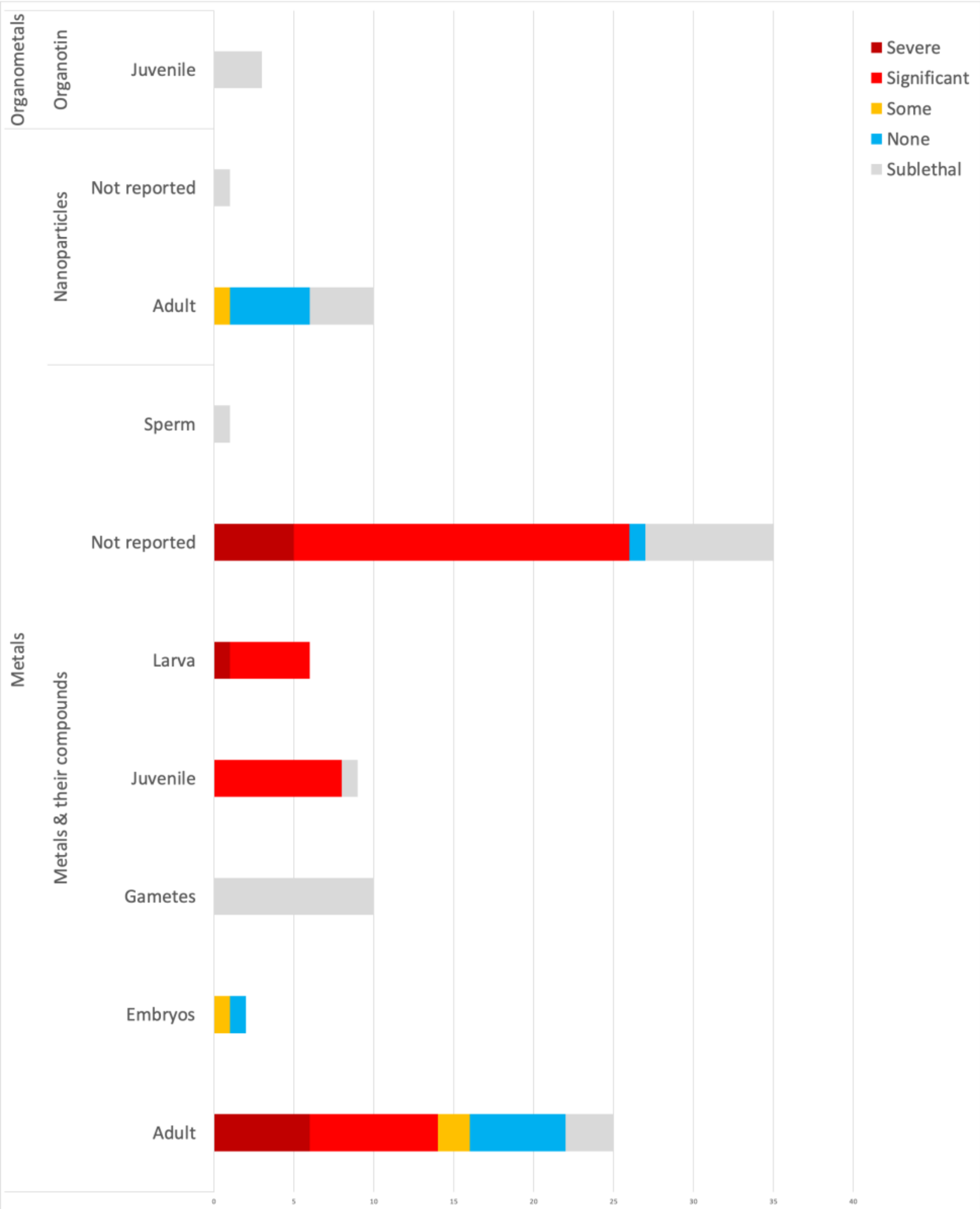

Figure 4.3. Count of ‘worst-case’ ranked mortalities due to exposure to metals, organometals and nanometals in the different life stages of selected polychaetes. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Bat & Raffaelli (1998) investigated the toxicity of copper, zinc and cadmium on the survival, emergence from sediment, number of casts and cast size (weight) of *Arenicola*, through 10-day sediment toxicity tests. Lungworms were exposed to sediments containing copper (1.7, 7, 14, 43, 87 µg/g), zinc (6.5, 23, 52, 73, 101 µg/g) or cadmium (<0.02, 9, 25, 48, 76 µg/g) for ten days. All control lugworms survived, however, the mortality of lugworms exposed to metals increased with increasing concentration.

At concentrations, of less than 7 µg/g copper, 23 µg/g zinc, and 9 µg/g cadmium no mortalities occurred. However, 100% mortality occurred at the highest tested concentrations of copper, zinc, and cadmium 87, 101 and 76 µg/g , respectively. Analyses showed copper to be more toxic to lugworms than zinc or cadmium, the LC50 values were 20, 50, and 35 µg/g, Cu, Zn and Cd, respectively.

All the *Arenicola* in the control sediment burrowed within 15 minutes of the start of the experiment and did not emerge throughout the bioassay. However, those exposed to contaminated sediment appeared. At concentrations, of less than 14 µg Cu, 52 µg Zn, and 25 µg Cd, lugworms were able to burrow in sediment. The number of casts in the control sediments was significantly different to the number from contaminated sediments, with significance increasing with concentration. At the higher concentrations of the metals, the size of the casts was reduced compared to those in the control sediment.

Bat *et al.* (2001) investigated the acute toxicity of zinc and lead to the polychaete *Hediste diversicolor*. Polychaetes of two size classes (20-30 mm in length, 125-250 mg in weight and 55-70 mm in length, 250-500 mg in weight) were exposed to a range of concentrations of the metals during 10-day and 28-day static bioassays. Mortality of the polychaetes increased with increasing concentration of the metals. Zinc was found to be more toxic than lead and smaller polychaetes were found to be more sensitive than larger polychaetes. For the 10-day bioassays, the LC50 values of small worms were 25,000 µg/l for zinc and 48,000 µg/l for lead. The larger worms had LC50 values of 35,000 µg/l for zinc and 65,000 µg/l for lead. In the 28-day bioassays, the LC50 values of small worms were 9,000 µg/l for zinc, and 19,000 µg/l for lead, while the larger worms had 28-day LC50 values of 18,000 µg/l for zinc and 28,000 µg/l for lead.

Bryan & Hummerstone (1973) investigated the adaptations of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. Worms from uncontaminated estuaries and worms from metal-contaminated estuaries were exposed to various concentrations of zinc (1, 2.5, 5, 10, 25, 100, 250 µg/ml) or cadmium (1, 2.5, 10, 25, 100 µg/ml) for up to 34 days to determine the time of survival at different concentrations. The worms from the contaminated sites had higher survival times than those from the uncontaminated sites. LC50 values for the uncontaminated worms exposed to zinc were 55 µg/ml, whereas the worms from the contaminated sites had LC50 values of 94 µg/ml. For worms from the uncontaminated sites, 100% mortality occurred in the 250, 100 and 25 µg/ml zinc treatments after 24, 48 and 382 hours of exposure, and 100% mortality occurred in the 25 and 100 µg/ml cadmium treatments after 406 and 233 hours, respectively.

Bryant *et al.* (1984) investigated the acute toxicity of chromium to three estuarine species (*Corophium volutator*, *Macoma balthica*, and *Nereis diversicolor*) at different temperatures (5, 10, and15°C) and a range of salinities (5 to 40%), at time intervals of up to 384 hours. Toxicity increased as temperature increased and as salinity decreased. *Nereis diversicolor* were exposed to chromium concentrations of 2, 4, 8, 16, 32, 64, and 128 ppm (+controls) and salinities of 5, 10, 15, 20, 25, 30, 35, and 40%. The 96-hour LC50 values ranged from 7.5 to 80 mg/l chromium depending on exposure temperature and salinity.

Burlinson & Lawrence (2007) used acute and chronic toxicity tests to investigate the temporal stability of a gradient in copper tolerance of *Hediste diversicolor* from the Fal estuary, Cornwall, UK. The study used toxicity testing to verify the existence of a gradient in tolerance along Restronguet Creek. Ragworms were collected from five locations along the Fal estuary and exposed to 4 mg/l copper. The acute toxicity test showed that the Mylor Creek ragworms were more sensitive (LT50 86 hours) and the tolerance of Restronguet Creek worms increased moving upstream from the mouth of the creek (LT50s 100–258 hours). Chronic toxicity testing was measured over 90 days of exposure at concentrations between 0.5 to 8 mg/l, using stepwise increases in concentration. Significant differences in tolerance were observed between populations.

Campbell *et al.* (2014) investigated the effects of ocean acidification and copper toxicity on the early life stages of the polychaete *Arenicola marina*. Sperm motility and velocity, fertilization success, and larval survival were assessed under the combined and singular effects of ocean acidification and copper exposure.

- Sperm were exposed to copper at 0.2, 2, and 20 µM at pH 8.28, 7.77, and 7.47 for ten minutes before being assessed for reductions in sperm swimming velocity and mobility. Exposure to copper or reduced seawater pH individually was observed to reduce sperm swimming velocity. Sperm swimming velocities were reduced at 2 and 20 µM copper in all three tested seawater pH. Sperm motility was reduced by up to 4% by exposure to copper concentrations of 2 and 20 µM. A 46% reduction in sperm motility was observed because of the combined stressors of copper and reduced pH.

- Male and female gametes were crossed under each of the experimental treatments (20 µM copper at pH 8.28 and 20 µM copper at pH 7.77) for 10 minutes to test fertilization success, before being washed and left to develop for 10 hours. Fertilization success was calculated as the percentage of viable postfertilization stages recorded from the total number of oocytes scored. Exposure to copper or reduced seawater pH individually, reduced fertilization success significantly. However, there was no further reduction in fertilization success under the combined stress of copper and reduced pH.

- Fertilized eggs were exposed to each of the experimental treatments (20 µM copper at pH 8.28 and 20 µM copper at pH7.77) for five days before the developmental stage and viability were assessed. Larval survival was calculated as the percentage of viable larvae from the post-fertilization stages scored. Exposure to copper or reduced seawater pH individually had no significant effect on larval survival. However, there was a strong significant interaction under the combined stress of copper and reduced pH, with a 24% reduction in larval survival after 5 days of exposure.

Casado-Martinez *et al.* (2008) investigated the suitability of lugworms (*Arenicola marina*) to study the bioaccumulation potential of Hg, PCB and PAH compounds from dredged sediments in laboratory exposures. During the study the lugworms were exposed to numerous sediment samples from several harbours that are important fishing and shipping ports near major centres of population, and, in areas mainly affected by historic mining activities but also hosting several industrial activities. The concentrations of Hg in sediments ranged between 0.05 and more than 136 mg/kg dry weight. A significant correlation between lugworm mortality and Hg concentration in sediments was observed up to 80% mortality. However, as the control sediment showed 15–20% mortality there was the potential that mortality could have been caused by other sediment conditions and not related to chemical stress. PAH and PCB sediment concentrations were not correlated with mortality.

Cong *et al.* (2011) investigated the impact of nano-, micro- and ionic-silver exposure on the polychaete, *Nereis diversicolor*. Ragworms were exposed to nano-, micro- and ionic-silver via sediment contamination at 0, 1, 5, 10, 25, and 50 ug/g dry weight sediment. The effects on survival, growth, DNA damage, and bioaccumulation were investigated. No concentration dependent mortality or growth effects were observed in any of the silver treatments.

Cong *et al.* (2014) investigated the toxicity of silver nanoparticles (Ag NPs, 20 and 80 nm) and aqueous Ag ($AgNO_3$) on the polychaete, *Nereis diversicolor*. Ragworms were exposed to silver via sediment contamination at 0, 5, 10, 25, 50, and 100 µg/g dry weight sediment for 10 days. The effects on survival, burrowing behaviour, bioaccumulation, lysosomal membrane stability, and DNA damage were investigated. No concentration dependent mortality was observed in any of the silver treatments. The burrowing tests were carried out after 10 days of exposure. Ragworms were placed in beakers containing natural clean sediment and filtered seawater, and worm burrowing positions were recorded every two minutes for 30 minutes and the time it took for the worms to become fully buried was recorded. The burrowing time of worms exposed to aqueous silver was comparable to the control. However, the burrowing time increased with higher exposure concentration. The form of silver affected the burrowing time significantly, and the exposure concentration had marginally significant effects on the burrowing time.

Cozzari *et al.* (2015) investigated the toxicity of sediments spiked with dissolved silver ($AgNO_3$), silver nanoparticles (63 ± 27 nm) and larger bulk silver particles (202 ± 56 mm), for up to 11 days at sublethal concentrations of 2.5, 5, 10 ug/g dry weight sediment. The effects on survival, bioaccumulation, and oxidative stress were investigated. Twenty-five worms were exposed to each form and concentration of silver for eleven days. No mortalities occurred in any of the treatments containing bulk silver particles. In the silver nitrate ($AgNO_3$) treatments, 8% of worms died at each of the concentrations after eleven days. Silver nanoparticles caused 8% and 16% mortalities in the 2.5 and 10 ug/g treatments after 4 days, respectively, and the 5 ug/g treatments caused 12% mortality by day seven. No significant worm weight or growth changes occurred from exposure to any of the silver treatments.

Durou *et al.* (2005) investigated the tolerance to metals and assessed the energy reserves in the polychaete *Nereis diversicolor* from clean and contaminated estuaries. The worms were exposed to various concentrations of copper (0, 0.316, 0.562, 10, 3.16, 5.62, 10 µM), cadmium (0, 100, 178, 316, 562, 1000 µM) and zinc (0, 31.6, 100, 178, 316, 562, 1000 µM) for up to three weeks at different salinities to establish the mean lethal time (LT50). No mortality occurred over the 21-day exposure period in any of the treatment groups at the lowest tested concentrations of zinc (31.6 and 100 µM).All the individuals died within 2 days at the highest concentration of zinc (1000 µM). No mortalities occurred in the control group at the lowest tested concentration of copper (3.16µM). However, the contaminated estuary group experienced 50% mortality by 12.6 days. The influence of worm size on the survival time was assessed in the zinc treatments. Larger worms had higher LT50s than smaller worms in the 178 and 316 µM treatment groups. However, there were no significant differences between survival time at 562 µM. The influence of salinity on the sensitivity of worms was also investigated. Almost all worms survived when exposed to 178 µM zinc at the lowest salinity of 15%, but at 25% salinity and 178 µM zinc 50% mortality occurred after 23 days in worms from the contaminated estuary and within16 days in worms from the control site. The difference in salinity did not cause differences in LT50s at the two higher tested concentrations 316 and 562 µM zinc. The mean lethal times (LT50s) of worms exposed to zinc from the contaminated estuary were higher than those from the clean control estuary, but the mean lethal times were not different when exposed to cadmium or copper. Larger worms were more tolerant to zinc exposure than smaller worms, and survival times were reduced at higher salinities.

Fernandez & Jones (1990) investigated the influence of salinity and temperature on the toxicity of zinc to *Nereis diversicolor*. Worms of two size classes (small and large) were exposed to a range of concentrations of zinc at different salinities for 96 hours to establish the 96-hour LC50 values. The LT50 values for worms exposed to 40 ppm zinc at five different salinities at 12°C were established. In addition, 96-hour LC50 values were determined for three different temperatures (6, 12 and 20°C). Zinc was the least toxic at 17.5% salinity and the 96-hour LC50 decreased with increasing temperature. The 96-hour LC50 values were 40, 32 and 9 ppm at 6, 12, and 20°C.

Fernandez & Jones (1990b) investigated the influence of salinity and temperature on the toxicity of zinc and copper to *Nereis diversicolor*. Worms were exposed to zinc at 38 ppm and copper at 0.5 ppm at different salinities (5, 17.5, and 30%) and temperatures (6, 12, and 20°C) for 96 hours. The LT50 values for the worms were established for each of the treatments. Both zinc and copper were the least toxic at 17.5% salinity. For all the treatments increases in temperature significantly increased the toxicity of the metals, with reduced LT50 values.

Foekeme *et al.* (2015) studied the effects of dissolved copper on a marine benthic and planktonic community in mesocosm experiments. The mesocosm experiments were run for 82 days at concentrations from 1 to 31 µg/l copper. The exposure to copper had clear effects on gastropod and bivalve molluscs, phytoplankton, zooplankton, sponges, and sessile algae. There were no indications that the lugworms were affected by the copper exposure. Across all of the treatments, survival rate was between 50 to 75% of the introduced individuals and the average dry weight of the individuals from the copper exposures was comparable to the controls. The lugworms did reproduce under the mesocosm exposures. However, juveniles were absent from at least one replicate of each concentration treatment, but no conclusions could be made.

Freitas *et al.* (2017) investigated the physiological and biochemical impacts induced by mercury and seawater acidification on *Hediste diversicolor*. The polychaetes were exposed for 28 days to 5 µg/l mercury and to 5 µg/l mercury at low pH (7.5). The effects on physiological alterations (respiration rate), biochemical markers related to metabolic potential, oxidative status, and survival were evaluated. The respiration rate tests showed that individuals exposed to stressful conditions (Hg and pH 7.5 + Hg) had higher mean respiration rate values than individuals from the control treatment. No mortality was observed in the control treatments. However, polychaetes exposed to Hg had 30% mortality, and polychaetes under the combination of both stressors (pH 7.5 + Hg) had 37% mortality.

Grant *et al.* (1989) investigated inherited metal tolerance of *Nereis diversicolor* by exposing field collected and laboratory bred worms from low and high metal sites to copper (0.5, 0.7, 10 µg/ml) and zinc (40 µg/ml) over 600+ hours to establish LT50 values. The LT50 values of the worms from the low metal sites were considerably lower than the LT50 values of those from the high metal sites. In addition, laboratory bred worms had noticeably lower tolerances than field derived worms.

Hateley *et al.* (1989) investigated the existence of heritable tolerance to heavy metals in estuarine populations. The survival times of the worms exposed to 0.5 and 0.7 mg/l copper ranged between 50 and 400 hours, and the survival time of those exposed to 40 mg/l zinc ranged between 100 and 300 hours depending on the population.

Jones *et al.* (1976) investigated the effects of salinity on the toxicity of copper to the polychaete *Nereis diversicolor*. The worms were exposed to copper concentrations at 0.0, 0.2, 0.4, and 0.8 µg/ml at a variety of salinities (5, 10, 17.5 and 34%) over 25 days. For each of the treatment groups, 96-hour LC50 values were calculated. At 5% salinity, the 96-hour LC50 was 0.2 µg/ml. At 10% salinity, the 96-hour LC50s were 0.44 and 0.45 µg/ml. At 17.5% salinity, the 96-hour LC50 was 0.48 µg/ml. At 34% salinity, the 96-hour LC50s were 0.37 and 0.45 µg/ml. The results showed that high and low salinities increased the toxicity of copper to the worms.

Mayor *et al.* (2008) investigated the toxicity of some treatments commonly used in salmonid aquaculture on *Hediste diversicolor*. The tested concentrations of copper and Emamectin benzoate had significant effects on the survival of the polychaetes with LC50 values of

74,987.96 µg/kg (wet sediment) for copper and 1367.71 µg/kg (wet sediment) for Emamectin benzoate.

McLeese & Ray (1986) investigated the toxicity of cadmium chloride and copper chloride on *Nereis virens*. The 144-hour LC50 for *Nereis virens* exposed to cadmium was calculated at 0.28 mg/l Cd. An LT50 value of 80 hours at 0.1 mg/l Cu was also determined.

Miramand & Unsal (1978) investigated the acute toxicity of vanadium (V) to *Nereis diversicolor*. The nine-day LC50 value was 10,000 µg/l V.

Moltedo *et al.* (2019) investigated the biological responses of the polychaete *Hediste diversicolor* to inorganic mercury exposure. Polychaetes were exposed to 0, 10, and 50 µg/l of mercury for 10 days following an adapted version of the acute toxicity test of ASTM E1611-00 2007. Effects on bioaccumulation, biomarkers of exposure, neurotoxicity, oxidative stress, genotoxicity, and cytochemistry were assessed. The mortality rate was <20% after the exposure period but was not significantly different from the control.

Moreira *et al.* (2005) investigated the effects of copper on the survival, growth, and feeding rates of the polychaete *Hediste* (*Nereis*) *diversicolor*. The 48-hour and 96-hour LC50s of copper were 241 and 125 µg/l, respectively.

Mouneyrac *et al.* (2003) investigated the trace-metal detoxification and tolerance of the estuarine worm *Hediste diversicolor* to zinc, cadmium, copper, and silver. Worms from the metal-contaminated area had higher tolerances to zinc, cadmium, and copper exposures with no mortality within seven days of exposure to copper and 96-hour LC50 values of approximately 400 µmol/l for zinc and 2250 µmol/l for cadmium. Whereas worms from the clean site that had 96-hour LC50 values of approximately 200 µmol/l for zinc, 500 µmol/l for cadmium and 15 µmol/l for copper. Worms from the clean site had a higher tolerance to silver than those from the contaminated site with a 96-hour LC50 of approximately 6 µmol/l compared to a 96-hour LC50 of 1 µmol/l for the worms from the contaminated site.

Ozoh & Jones (1990) investigated the effects of copper exposure, temperature, and salinities on the survival of 1-day and 7-day-old *Hediste* (*Nereis*) *diversicolor* larvae. One-day-old larvae were exposed to 5, 10, and 20 µg/l copper, and seven-day-old larvae were exposed to 20, 40, 80, and 100 µg/l copper at various temperatures (12, 17, and 22°C) and salinities (7.6, 15.2, 22.9, and 30.5%) for 96 hours. Mortality was recorded across all the treatments and was found to be influenced by salinity, temperature, and copper. The one-day-old larvae were more sensitive to copper than the older larvae.

Ozoh (1992) investigated the effects of copper exposure, temperature, salinity, and sediment on the survival of juvenile *Hediste* (*Nereis*) *diversicolor*. Mortality was recorded across all of the treatments. Without sediment, increasing salinity and increasing temperature reduced the toxicity of copper, and the LC50 values ranged from 247 to 512 µg/l. However, with sediment, increasing temperature and increasing salinity increased the toxicity of copper to the worms, and the LC50 values ranged from 2700 to 4100 µg/l.

Rasmussen & Andersen (2000) investigated the effects of cadmium on the lugworm, *Arenicola marina*. The lugworms were exposed to cadmium for short term 24-hour exposures and to long-term 30-day exposures at a variety of salinities (10%, 15% and 20%). Exposure to cadmium increased the lugworm's sensitivity to hypo-osmotic stress. Long-term exposures to 0.1, 1, and 10 µg/l caused 15%, 30%, and 100% mortality, respectively. In the short-term exposures, mortality did not occur in the 0.1 and 1 µg/l cadmium treatments at 15% salinity, but at 20% salinity, 13% mortality occurred at 0.1 µg/l cadmium treatment. In the treatments where the salinity was altered from 20 to 10% increased mortality occurred at 0.1 and 1 µg/l cadmium with 25% and 50% mortality, respectively.

4.2 Organometals

Only two articles assessed the exposure of organotins on polychaetes. Walsh *et al.*, 1986 was not accessible and the data for was retrieved from ECOTOX.

Beaumont *et al.* (1989) conducted a four-month microcosm experiment to investigate the toxicity of tributyltin (TBT) on marine organisms. The setup contained a series of sandy-substratum flow through microcosms containing two bivalve species (Cerastoderma edule and *Scrobicularia plana*), two polychaete species (*Nereis diversicolor* and Cirratulus cirratus), a crustacean (*Corophium volutator*) and a gastropod (Littorina littorea). TBT was introduced into three microcosms at high (1-3 µg/l) concentrations and three microcosms at low (0.06-0.17 µg/l) concentrations. High mortalities of *Nereis diversicolor* were recorded in all microcosms including the control. In the control and low-level TBT treatments, up to 16 species of non-introduced invertebrates were found, whereas, in the high TBT treatments, only two additional juvenile bivalves were observed. Non-introduced *Nereis diversicolor*, *Arenicola marina*, and *Eteone* spp. occurred in the low-level TBT and the control treatments but not in the high-level TBT treatments.

4.3 Nanoparticulate metals

Ten articles examined the effects of nanoparticulate (NP) metals on polychaetes. The evidence is summarized below.

Buffet *et al.* (2011) investigated the effects of copper oxide nanoparticles on the behavioural and biochemical responses of *Scrobicularia plana* and *Hediste diversicolor*. The polychaetes were exposed to either 10 µg/l copper oxide nanoparticles, 10 µg/l dissolved copper or natural seawater only (control) for seven days. The feeding and burrowing behaviour of the polychaetes were assessed using 20 individuals from each treatment. After four days of exposure, the burrowing tests were carried out by placing the ragworms onto sediment and recording their positions every two minutes for 30 minutes. The feeding tests were carried out after seven days of exposure. The ragworms were exposed to Artemia salina larvae for an hour after which time the remaining larvae were collected and counted to establish a feeding rate per individual. The results from the burrowing test showed that the ragworms exposed to soluble copper had reduced burrowing times compared to the controls. However, no significant difference was seen between those exposed to the nanoparticulates and the control. The feeding rate of the ragworms was not affected by either form of copper.

Buffet *et al.* (2012) investigated the effects of zinc oxide nanoparticles (NPs) in sediment on the feeding rate and burrowing behaviour of the ragworm *Hediste diversicolor*. Three treatments were conducted: in natural seawater only; diethylene glycol (DEG) alone, (at the same concentration as with NPs) and zinc oxide nanoparticles (3 mg/kg sediment) in DEG. The burrowing tests were carried out after eight days of exposure to one of the three treatments. Twenty ragworms were placed on sediment and their positions were recorded every two minutes for 30 min. The feeding rate of the ragworms was estimated following 11 days of exposure to the treatments. The feeding rate was established by calculating the number of *Artemia salina* larvae ingested per hour per individual. Exposure to zinc oxide nanoparticles reduced both the burrowing time and feeding rates of the ragworms.

Buffet *et al.* (2013) investigated the effects of copper oxide nanoparticulates and copper nitrate on the feeding rate and burrowing behaviour of the ragworm *Hediste diversicolor* in environmentally realistic conditions in outdoor mesocosms. The burrowing and feeding rate tests were carried out after 7 and 14 days of exposure to 10 µg/l copper. Ragworm behavioural toxicity tests were carried out using a multi-freshwater biomonitoring to record the ragworms movements over a 7-hour period. The feeding rate was established by calculating the number of *Artemia salina* larvae ingested per hour per individual. Exposure to copper oxide nanoparticulates and copper nitrate affected the burrowing behaviour and feeding rates of the ragworms significantly following 14 days of exposure.

Buffet *et al.* (2014) investigated the effects of silver nanoparticulates and silver nitrate on the feeding rate and burrowing behaviour of the ragworm *Hediste diversicolor* and clam *Scrobicularia plana* in environmentally realistic conditions in outdoor mesocosms. The burrowing and feeding rate tests were carried out after 21 and 14 days of exposure to silver at 10 µg/l. The feeding rate was established at day 14 by calculating the number of *Artemia salina* larvae ingested per hour per individual. The burrowing tests were carried out after 21 days of exposure. Ragworms were placed on to sediment surface and the number of burrowed individuals was recorded every two minutes for 30 min. The feeding rate of the ragworms was not affected by 10 µg/l of silver in either form (nanoparticulate or ionic); neither was burrowing affected by silver nanoparticles. However, soluble silver significantly affected burrowing.

Cong *et al.* (2011) investigated the impact of nano-, micron- and ionic-silver exposure on the polychaete, *Nereis diversicolor*. Ragworms were exposed to nano-, micron- and ionic-silver via sediment contamination at 0, 1, 5, 10, 25, and 50 µg/g dry weight sediment. The effects on survival, growth, DNA damage, and bioaccumulation were investigated. No concentration dependent mortality or growth effects were observed in any of the silver treatments.

Cong *et al.* (2014) investigated the toxicity of silver nanoparticles (Ag NPs, 20 and 80 nm) and aqueous $AgNO_3$ on *Nereis diversicolor*. Ragworms were exposed to silver via sediment contamination at 0, 5, 10, 25, 50, and 100ug/g dry weight sediment for 10 days. The effects on survival, burrowing behaviour, bioaccumulation, lysosomal membrane stability, and DNA damage were investigated. No concentration dependent mortality was observed in any of the silver treatments. The burrowing tests were carried out after 10 days of exposure. Ragworms were placed in beakers containing natural clean sediment and filtered seawater, and worm burrowing positions were recorded every 2 two minutes for 30 minutes and the time it took for the worms to become fully buried was recorded. The burrowing time of worms exposed to aqueous silver was comparable to the control. However, the burrowing time increased with higher exposure concentration. The burrowing time increased with higher exposure to silver nanoparticles. The form of silver significantly affected the burrowing time, and the exposure concentration had marginally significant effects on the burrowing time.

Cozzari *et al.* (2015) investigated the toxicity of sediments spiked with dissolved silver ($AgNO_3$), silver nanoparticles (63 ± 27 nm) and larger bulk silver particles (202 ± 56 mm), for up to 11 days at sublethal concentrations of 2.5, 5, 10 µg/g dry weight sediment. The effects on survival, bioaccumulation, and oxidative stress were investigated. Twenty-five worms were exposed to each form and concentration of silver for eleven days. No mortalities occurred in any of the treatments containing bulk silver particles. In the $AgNO_3$ treatments, 8% of worms died at each of the concentrations after eleven days. Silver nanoparticles caused 8% and 16% mortalities in the 2.5 and 10 µg/g treatments after 4 days, respectively, and the 5 µg/g treatments caused 12% mortality by day seven. No significant worm weight or growth changes occurred from exposure to any of the silver treatments.

Galloway *et al.* (2010) investigated the sublethal toxicity of nano-titanium dioxide and carbon nanotubes on the marine polychaete *Arenicola marina*. The lugworms were exposed through natural sediments to a 10-day OECD/ASTM 1990 acute toxicity test. Sediment was prepared with either single-walled carbon nanotubes (0.003–0.03 g/kg), nano-titanium dioxide (1–3 g/kg), or seawater alone. The lugworms were fed every other day and feeding behaviour was monitored every 48 hours. Casts were collected, dried overnight and weighed, with seawater renewals every 48 hours following cast collection. After 10 days of exposure, the lugworms were removed from the exposure sediment and the lugworm's ability to re-bury into clean sediment was assessed following the OECD/ICES A. marine burrowing bioassay. Sediment exposure to single-walled carbon nanotubes or nano-titanium dioxide had no effects on the burrowing behaviour of the lugworm. During the exposure period, single-walled carbon nanotubes had no effects on the feeding behaviour of the lugworms. However, nano-titanium dioxide exposure caused a significant impact on feeding behaviour with reductions in casting rate at 2g/kg nano-titanium dioxide.

Thit *et al.* (2015) investigated the bioaccumulation, subcellular distribution, and toxicity of sediment-associated copper in the ragworm *Nereis diversicolor*. The ragworms were exposed to sediment spiked with aqueous copper, copper oxide nanoparticulates, or copper oxide micro-particles at 150 µg Cu /g dwt sediment for 10 days. The exposure caused 62.5% mortality for ragworms exposed to aqueous copper, 37.5% mortality to ragworms exposed to copper oxide micro-particles, and 0% mortality after exposure to copper oxide nanoparticles. There was positive weight gain for the control and copper oxide nanoparticulate-exposed ragworms; whereas there was weight loss for the ragworms exposed to aqueous copper and copper oxide micro-particles. However, the weight loss was not significant. In the burrowing assay, the control ragworms and the copper oxide micro-particle-exposed ragworms burrowed quickly into the sediment, whereas the ragworms exposed to aqueous copper and copper oxide nanoparticulates had significantly longer burrowing times of 19.3 and 12.2 hours compared to the controls that had a mean burrowing time of 0.12 hours.

Wang *et al.* (2014) investigated the toxicity, bioaccumulation, and biotransformation of silver nanoparticulates in marine organisms. *Nereis virens* was exposed to a 28-day bioaccumulation study via sediment contamination in a flow through system. Sediments were contaminated with 7,500 µg/kg dry-weight sediment of either silver nitrate, citrate silver nanoparticulates or polyvinyl-pyrrolidone-coated silver nanoparticulates. No mortality occurred in any of the treatments throughout the 28-day study. However, weight loss did occur in all of the treatments.

4.4 Sensitivity assessment – Transitional metals and organometals

The count of ranked mortalities due to 'Transitional metals and organometals ' are summarized in Figure 4.1 above and Table 4.1 and below. The data presented in Table 3.1 include all life stages and articles where life stage were not reported or were unspecified (NR). The majority of the effects of exposure to 'Metals' reported (59%) resulted in mortality, 13% in 'no' mortality and 28% in sublethal effects (Table 4.1 and Figure 4.1).

Table 4.1. Summary of count of 'worst-case' ranked mortalities to 'Metals and Organometals' contaminants reported in the evidence review and resultant proposed sensitivity assessments for polychaete species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Species name	Group/Type	Chemical name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Arenicola marina											
	Metals										
		Cadmium	**2**					2	N	M	M
		Copper	**1**		1	2	2	6	N	M	M
		Mercury	**1**					1	N	M	M
		Zinc	**1**					1	N	M	M
	Nanoparticles										
		Titanium					**1**	1	H	H	NS
	Organotin										

Species name	Group/Type	Chemical name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
		Tributyltin (TBT)					**1**	1	H	H	NS
Total			**5**		**1**	**2**	**4**	**12**			
Hediste diversicolor											
	Metals										
		Cadmium		**1**		1		2	L	H	L
		Copper	**1**	7			13	21	N	M	M
		Lead		**1**				1	L	H	L
		Mercury		**1**	1		1	3	L	H	L
		Silver		**1**			1	2	L	H	L
		Zinc		**2**				2	L	H	L
	Nanoparticles										
		Copper					**2**	2	H	H	NS
		Silver					**1**	1	H	H	NS
		Zinc					**1**	1	H	H	NS
Total			**1**	**13**	**1**	**1**	**19**	**35**	**N**	**NL**	**H**
Nereis diversicolor											
	Metals										
		Cadmium	**1**	2				3	N	M	M
		Chromium		**1**				1	L	H	L
		Copper	**2**	10			2	14	N	M	M
		Lead		**1**				1	L	M	M
		Silver			**1**	4		5	M	H	L
		Vanadium		**1**				1	L	H	L
		Zinc	**3**	6			1	10	N	M	M
	Nanoparticles										
		Copper				1		1	H	H	NS
		Silver			**1**	2		3	M	H	L
	Organotin										
		Tributyltin (TBT)					**1**	1	H	H	NS
Total			**6**	**21**	**2**	**7**	**4**	**40**			
Nereis virens											
	Metals										
		Cadmium		**2**				2	L	H	L

Species name	Group/Type	Chemical name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
		Chromium		**1**				1	L	H	L
		Copper		**3**				3	L	H	L
		Lead		**1**				1	L	H	L
		Silver				**1**		1	H	H	NS
		Zinc		**1**				1	L	H	L
	Nanoparticles										
		Silver				**2**		2	H	H	NS
Total				**8**		**3**		**11**			
Nephtys caeca											
	Metals										
		Cadmium					**1**	1	**H**	**H**	**NS**
***Eteone* spp.**											
	Organotin										
		Tributyltin (TBT)					**1**	1	**H**	**H**	**NS**
***Tubificoides* spp.**											
	Metals										
		Copper					**2**	2	**H**	**H**	**NS**
Total			**12**	**42**	**4**	**13**	**30**	**102**			

Overall, the effects of metal exposure vary depending on the type of metal, its chemical form (e.g., salt), the environmental conditions (e.g., pH, salinity, and temperature), and the concentration and duration of exposure. The effects also vary between species, depending on their habitat (e.g., sedimentary), feeding mechanism (i.e., the method of ingestion of the metals), and any metal detoxification pathways the species possess. In general, for estuarine animals heavy metal toxicity increases as salinity decreases and temperature increases (McLusky *et al.*, 1986). Bryan (1984) reported that short-term toxicity in polychaetes was highest to Hg, Cu, and Ag, declined with Al, Cr, Zn, and Pb with Cd, Ni, Co, and Se being the least toxic. It was reported that polychaetes have a range of tolerances to heavy metal levels of Cu, Zn, As, and Sn being in the order of 1500-3500 µg/g. An analysis of organisms from Restronguet Creek revealed that *Nephtys hombergii* from the middle and lower reaches of the creek contained appreciably higher concentrations of Cu (2227 µg/g dry wt), Fe, and Zn than comparable specimens of *Hediste diversicolor*.

In addition, there is evidence that some polychaete species can adapt to metal contamination in the long-term (Bryan & Hummerstone, 1971, 1973; Grant *et al.*, 1989; Hateley *et al.*, 1989; Mouneyrac *et al.*, 2003; Alla *et al.*, 2006b; Burlinson & Lawrence, 2007; McQuillan *et al*., 2014). For example, Rainbow & Phillips (1993) noted that *Hediste (Nereis) diversicolor* was able to regulate its tissue concentration of several trace elements including manganese and zinc. McQuillan *et al*. (2014) suggested that some species (*Nephtys hombergii*) had developed metal-resistant populations as a functional genetic trait to copper homeostasis.

4.4.1 *Arenicola* spp.

Bat & Raffaelli (1998) reported 100% mortality of *Arenicola marina* exposed to the highest tested concentrations of copper, zinc, and cadmium 87, 101 and 76 µg/g, respectively. Campbell *et al.* (2014) investigated the effects of ocean acidification and copper toxicity on the early life stages of the polychaete *Arenicola marina*. Sperm motility was reduced by up to 4% by exposure to copper concentrations of 2 and 20 µM. And further reduced by 46% because of the combined stressors of copper and reduced pH. Exposure to copper or reduced seawater pH individually, reduced fertilization success significantly. Exposure to copper or reduced seawater pH individually had no significant effect on larval survival. However, there was a strong significant interaction under the combined stress of copper and reduced pH, with a 24% reduction in larval survival after five days of exposure.

Casado-Martinez *et al.* (2008) reported a significant correlation between lugworm mortality and Hg concentration in sediments with up to 80% mortality but as the control sediment showed 15–20% mortality there was the potential that mortality could have been caused by other sediment conditions and not related to chemical stress. Rasmussen & Andersen (2000) reported that cadmium contamination increased the susceptibility of *Arenicola marina* to hypo-osmotic stress.

Overall, the evidence suggests that *Arenicola marina* can experience severe mortality due to exposure to copper, cadmium, mercury, and zinc. **Therefore, its resistance to 'transitional 'metals' is assessed as 'None', resilience as 'Medium', and sensitivity as 'Medium'**.

The evidence on the effects of Tributyltin was limited to two papers, one of which was not accessible (Walsh *et al.*, 1984). Beaumont *et al.* (1989) TBT was introduced into three microcosms at high (1-3 µg/l) concentrations and three microcosms at low (0.06-0.17 µg/l) concentrations. Non-introduced *Nereis diversicolor*, *Arenicola marina*, and *Eteone* sp. occurred in the low-level TBT and the control treatments but not in the high-level TBT treatments. Overall, only sublethal effects were reported in the evidence reviewed. **Therefore, the sensitivity of *A. marina* to TBT is assessed as 'Not sensitive'** but with 'Low' confidence due to the lack of evidence.

Similarly, the evidence for the effects of nanoparticulate metals, that is, nanoparticulate titanium was limited to a single study (Galloway *et al.*, 2010). Only sublethal effects were reported in the evidence reviewed. **Therefore, the sensitivity of *A. marina* to nanoparticulate titanium is assessed as 'Not sensitive'** but with 'Low' confidence due to the lack of evidence.

4.4.2 *Hediste* spp. and *Nereis* spp.

Hediste spp., and *Nereis* spp. (often as *Hediste* (*Nereis*) *diversicolor*) were the most studied genera and contributed 73.5% of the results on the effects of 'transitional metals and organometals' in the evidence reviewed. As noted above, the effect of 'metal' exposure varied between studies depending on the metal and its chemical form, and the environmental conditions used in the study. Nevertheless, exposure to 'metal' contaminants was reported to resulted in 'Severe' mortality in 8% of the results from *Hediste* (*Nereis*) *diversicolor* and *Nereis virens*. 'Significant' mortality was reported in 48.8% of the results but sublethal effects were reported in 26.7% of the results. Overall, the most toxic metals (in terms of the reported mortality) were copper, zinc, cadmium, lead, mercury, and silver. Exposure to vanadium was also reported to result in 'significant' mortality but in a single study.

In *Hediste diversicolor,* the acute toxicity is dependent on the rate of uptake of the metal since this determines the speed with which the lethal dose is built up. The rate of intake is important because this determines whether the organism's detoxification mechanisms can regulate internal concentrations. The resistance of *Hediste diversicolor* is thought to be dependent on a complexing system which detoxifies the metal and stores it in the epidermis and nephridia (Bryan & Hummerstone, 1971; McLusky *et al.* 1986). *Hediste diversicolor* has been found successfully living in estuarine sediments contaminated with copper ranging from 20 µM Cu/g in low copper areas to >4000 µM Cu/g where mining pollution is encountered e.g. Restronguet Creek, Fal Estuary, Cornwall (Bryan & Hummerstone, 1971). Attempts to change the tolerance of different populations of *Hediste diversicolor* to different sediment concentrations of copper have shown that it is not readily achieved, which suggests that increased tolerance to copper has a genetic basis (Bryan & Hummerstone, 1971; Bryan & Gibbs, 1983). Since juveniles remain in the infauna throughout their development, selection for metal tolerance can be expected to be operative from an early stage (Bryan & Gibbs, 1983).

Overall, the evidence suggests that the **resistance of *Hediste* spp. and *Nereis* spp. ranges from 'None' to 'Low' for most of the 'transitional metals' examined, except silver**. **Therefore, as resilience is probably 'Medium', the 'worst-case' sensitivity is assessed as 'Medium'.** However, the toxicity of metals varies with the environmental conditions, and local populations can adapt to long-term contamination.

As above, the evidence on the effects of tributyltin was limited to two papers, one of which was not accessible (Walsh *et al.*, 1984). Beaumont *et al.* (1989) introduced TBT into three microcosms at high (1-3 µg/l) concentrations and three microcosms at low (0.06-0.17 µg/l) concentrations. High mortalities of *Nereis diversicolor* were recorded in all microcosms including the control, so the results were inconclusive. Overall, only sublethal effects were reported in the evidence reviewed. **Therefore, the sensitivity of *Nereis* spp. to TBT is assessed as 'Not sensitive'** but with 'Low' confidence due to the lack of evidence.

Sublethal effects and 'no' mortality were reported in all but one of the studies reviewed that examined the effects of nanoparticulate metals on polychaetes (Table 4.1). The nanoparticulates did result in reduced burrowing speed or activity, where an effect was reported. While a reduction in burrowing ability may reduce feeding or increase the susceptibility to predation, 'no' direct mortality was reported. However, Cozzari et al. (2015) reported that silver nanoparticles caused 8% and 16% mortalities in the 2.5 and 10 µg/g treatments after 4 days, respectively, and the 5 µg/g treatments caused 12% mortality by day seven. **Therefore, the worst-case resistance of *Nereis* spp. to nanoparticulate metals is assessed as 'Medium', resilience as 'High' and sensitivity as 'Low'** but with 'Low' confidence due to the lack of evidence.

4.4.3 *Nephtys* spp., *Eteone* spp. and *Tubificoides* spp.

Nephtys hombergii from the middle and lower reaches of Restronguet Creek contained appreciably higher concentrations of Cu (2227 µg/g dry wt), Fe and Zn than comparable specimens of *Hediste diversicolor* (as *Nereis diversicolor*). However, amongst polychaetes within the creek, there was evidence that some metals were regulated. In *Nephtys hombergii* the head end of the worm became blackened and X-ray microanalysis by Bryan & Gibbs (1983) indicated that this was caused by the deposition of copper sulphide in the body wall. In the same study, Bryan & Gibbs (1983) presented evidence that *Nephtys hombergii* from

Restronguet Creek possessed increased tolerance to copper contamination. Specimens from the Tamar Estuary had a 96-hour LC50 of 250 µg/l, whilst those from Restronguet Creek had a 96-hour LC50 of 700 µg/l (35 psu; 13°C). Bryan & Gibbs (1983) suggested that since the area had been heavily contaminated with metals for over 200 years, there had been adequate time for metal-resistant populations to develop especially for relatively mobile species. McQuillan *et al*. (2014) suggested that some species (*Nephtys hombergii*) had developed metal-resistant populations as a functional genetic trait to copper homeostasis. **Therefore, the resistance of *Nephtys* spp. to 'transitional metals and organometals' is assessed as 'Low', resilience as 'High' and sensitivity as 'Low' but with 'Low' confidence as it is based on a single study.**

The evidence on the remaining species is limited to single observations from four studies. In all cases, only sublethal results were reported. The **sensitivity of *Eteone* spp. and *Tubificoides* spp. to 'Transitional metals and organometals' is assessed as 'Not sensitive'** but with 'Low' confidence due to the lack of evidence.

5 Synthetic compounds – including Pesticides and Pharmaceuticals

A total of 47 results (ranked ‘worst-case’ mortalities) were obtained from 25 articles that examined the effects of ‘Synthetic compounds’ on polychaete species. Pharmaceuticals were most studied, with 51% of the results, followed by Pesticides/biocides with 40% followed by ‘Synthetics (other)’ with 6% of the results and Polychlorinated biphenyls (PCBs)’ with 2% (Figure 5.1). *Hediste (Nereis) diversicolor* was the most studied species with 46.8% of the reported results (mainly under pharmaceuticals), followed by *Arenicola marina* with 38.3% of the results overall. *Arenicola cristata* was reported in four studies of pesticides/biocides, and represented only 8.5% of the results. *Eteone longa, Nephtys* spp., and *Streblospio benedicti* were only reported once throughout the 25 selected articles.

5.1 Pesticides/biocides

Where possible the pesticides/biocides were categorised by their function or target, for example herbicides, insecticides, rodenticides, or arcarides. A total of 19 results (ranked ‘worst-case’ mortalities) were obtained from 14 articles (Figure 5.2). The evidence was limited with the effects of organophosphates, organohalogens, carbamates, and parasiticides were reported in four or less results and other pesticide/biocide types in less than two results. *Hediste* (*Nereis*) *diversicolor* dominated the studies with 47% of the results, followed by *Arenicola* sp. with 37%. The studies reported ‘Severe’ mortality in 16% of the results, ‘Significant’ mortality in 31.5%, ‘Some’ mortality in 5%, and ‘No’ mortality in 16% of the results while the remaining 31.5% results reported sublethal effects. The life stages examined in pesticide/biocide exposures were either adults (47% of the results) or not reported (53% of the results). The evidence is summarized below.

Allen *et al.* (2007) exposed *Arenicola marina* to Ivermectin for either 10 days for acute toxicity testing or for 100 days of chronic testing. Ivermectin concentrations for the 10-day acute tests ranged from 2 to 44 µg IVM/kg wet sediment. For the 100-day test, concentrations ranged from 0.5 to 8 µg IVM/kg wet sediment. In the acute toxicity testing, Ivermectin had a significant effect on the survival of the lugworms; the 10-day LC50 was 17.9 µg IVM/kg wet sediment in Test 1 and 14.8 µg IVM/kg wet sediment in Test 2. In addition, the mean daily casting rates declined with increasing Ivermectin concentration.

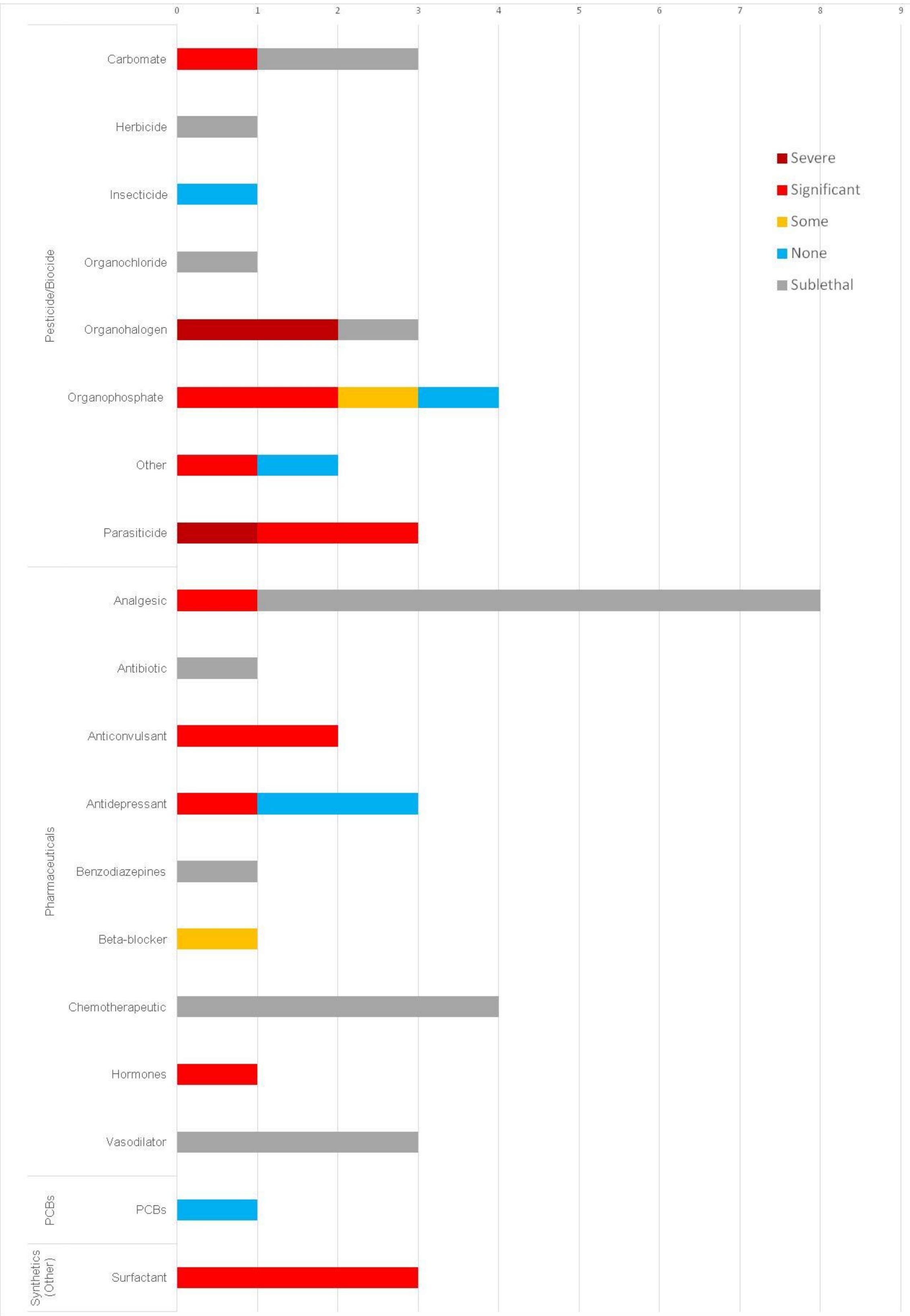

Figure 5.1. Count of worst-case ranked mortalities due to exposure to Synthetic compounds in selected polychaetes. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

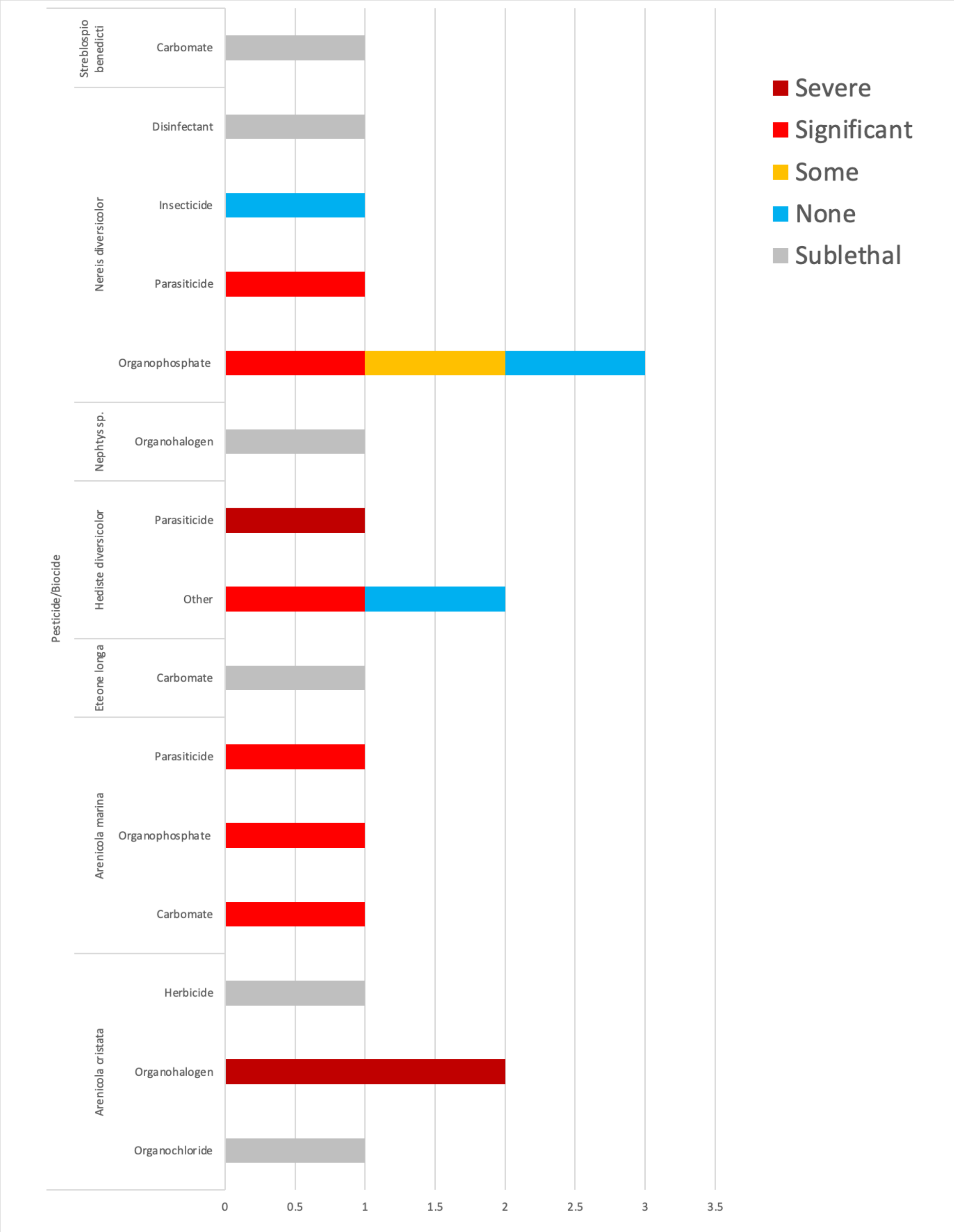

Figure 5.2. Count of worst-case ranked mortalities due to exposure to 'Pesticide/Biocides' in selected polychaete species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

In Test 1, the 10-day LOEC and NOEC were 5.0 and 3.0 µg IVM/kg respectively and the 10-day EC50 was 6.5 µg IVM/kg. In Test 2, the casting LOEC and NOEC were 4.0 and 2.0 µg IVM/kg respectively and the 10-day EC50 was 5.5 µg IVM/kg. In the chronic tests, the control survival in the *A. marina* long-term test was 100%. However, the exposed lungworms had a 100-day LC50 of 6.8 µg IVM/kg. The mean casting rate was significantly reduced at all concentrations, with a LOEC of 0.5 µg IVM/kg and a NOEC of <0.5 µg IVM/kg. The 100-day EC50 for casting was calculated at 7.4 µg IVM/kg.

Collier & Pinn (1998) investigated the impact of the sea lice treatment Ivermectin on a benthic community. *Hediste diversicolor* and the other test species were exposed to Ivermectin at concentrations of 0.8, 8.0, and 80 mg/m^2 for a total of 21 days. Significant differences in the abundance and biomass of the benthic community were observed. As the concentration of Ivermectin increased, the impacts on the benthic community changed more rapidly. *Hediste diversicolor* was reported to be the most sensitive of the species of those included in the experiment, as 8.0 mg/m^2 Ivermectin caused 100% mortality within 14 days.

Conti (1987) investigated the acute toxicity of three detergents and two insecticides in the lugworm, *Arenicola marina*. Two anionic detergents: sodium dodecyl benzene sulfonate, and sodium dodecyl sulphate; one non-ionic detergent: Triton X-100; one carbamate insecticide, Carbaryl, and one organophosphate insecticide, Parathion- ethyl were used. For each toxicity test, five dose levels and one control with 10 lugworms for each dose level and control were used. The 48-hour LC50 values for each substance were determined by the probit analysis method (Bliss, 1935). The 48-hour LC50s of the two anionic detergents were 15,200 µg/l for sodium dodecyl benzene sulfonate and 12,500 µg/l for sodium dodecylbenzenesulfonate. The 48-hour LC50 of the non-ionic detergent Triton X-100 was 15,200 µg/l and the 48-hour LC50s of the two pesticides were 2,700 µg/l for Parathion-ethyl and 7,200 µg/l for Carbaryl.

Dumbauld *et al.* (2001) observed the effects of the application of the pesticide Carbaryl on the estuarine benthic community in oyster culture sites. The experiment had four sets of replicate treatment and control plots at each of two sites located in the Palix River sub-estuary and Cedar River sub-estuary. The abundance of benthic community organisms was determined after (24 hours, 2 weeks, 1 month and 1 year) the application of Carbaryl at 5.6 kg/ha. The results showed no trends and no significant differences in the abundance of the polychaetes *Streblospio benedicti* and *Eteone longa*.

Garnas *et al.* (1977) exposed *Arenicola cristata* to 1000 µg/l 14C-Kepone for 5 days. Exposure was lethal causing 100% mortality.

Grant & Briggs (1998) undertook toxicity tests to establish the toxicity values of Ivermectin to numerous estuarine and marine invertebrates. The toxicity tests for *Nereis diversicolor* were conducted over 96 hours. The concentrations calculated to cause 10% and 50% mortality of individuals within 96 hours were 5.4 and 7.75 µg/l.

Mayor *et al.* (2008) investigated the toxicity of some treatments commonly used in salmonid aquaculture on *Corophium volutator* and *Hediste diversicolor* in whole sediment bioassay tests. *Hediste diversicolor* was exposed to copper ($CuSO_4$) and Slice® (Emamectin benzoate) for 10 days. The tested concentrations of copper and Emamectin benzoate had significant effects on the survival of the polychaetes with LC50 values of 74,987.96 µg/kg (wet sediment) for copper and 1,367.71 µg/kg (wet sediment) for Emamectin benzoate.

McBriarty *et al.* (2018) investigated the short-term effects of the anti-sea lice therapeutant emamectin benzoate on *Nereis virens* in sediment bioassay tests. The polychaetes were exposed to emamectin benzoate for 30 days at 400 µg/kg dry weight. The tested concentrations of emamectin benzoate had no significant effects on the survival of the polychaete. However, polychaete mass and burrowing behaviour changed. The specific growth rates of the polychaetes decreased over time in the emamectin benzoate exposure treatment, with significant differences from the control. In the emamectin benzoate exposure treatment, more than 50% of the polychaete emerged from their burrows over the first 20 days and few or none were burrowed in the final 10 days of the experiment, while between 58 and 100% of the polychaetes remained burrowed over the entire experiment.

Rubinstein (1978) investigated the influence of sodium pentachlorophenate on the feeding behaviour of *Arenicola cristata*. Worms were exposed to sodium pentachlorophenate at 45, 80, 156, and 276 µg/l for 144 hours. Significant reductions in the feeding behaviour of the worms occurred at 80 µg/l and above.

Rubinstein (1979) investigated the influence of the pesticide Kepone on the feeding behaviour of *Arenicola cristata*. Worms were exposed to Kepone at 2.8, 4.5, 6.6, 7.4, and 29.5 µg/l for 144 hours. The highest tested concentration of Kepone was toxic to the worms causing 100% mortality. No mortalities occurred at the lower tested concentrations. Significant reductions in the feeding behaviour of the worms occurred at all tested concentrations between 60 to 144 hours.

Scanes *et al.* (1993) observed the effects of a spillage of the pesticide Aldrin on the biota of an estuarine beach, after an industrial accident in Hardys Bay, New South Wales. Water and sediment samples were taken three weeks following the spill, to measure the level of contamination caused by the spill. In addition, the abundance of some intertidal biota was determined at the site of the spillage and in other uncontaminated areas. The samples of water taken at the spill site were not contaminated by Aldrin three weeks post-spill; however, the sediment samples contained Aldrin. The abundance of polychaetes was not significantly different when compared to the uncontaminated sites, but the abundance of Crustacea was greatly reduced.

Scaps *et al.* (1997) investigated the effects of organophosphate and carbamate pesticides on acetylcholinesterase and choline acetyltransferase activities of the polychaete *Nereis diversicolor*. Ragworms were exposed to three organophosphate pesticides, Malathion, Parathion-ethyl and Phosalone, and one carbamate pesticide Carbaryl for up to 21 days at concentrations between 10^{-6} and 10^{-12} M. There was 100% survival in all of the treatments until day 14 of the exposures. However, by day 21, 20% of worms exposed to 10^{-6} and 10^{-8} M Malathion died, and 33.5 and 62.5% mortality occurred in the 10^{-8} and 10^{-6} M parathion-ethyl treatments.

Schoor & Newman (1976) exposed *Arenicola cristata* to the pesticide Mirex to study behavioural changes. The worms were exposed to Mirex for 30 days. Mirex concentrations in the water raged between 0.016 to 0.062 µg/l during the exposure period. Statistically significant changes in the feeding and burrowing behaviour of the worms were observed from exposure to Mirex when compared to the controls.

Underwood & Paterson (1993) examined the effect the weekly application of the biocide formaldehyde to the sediment surface (at 200 ml/m^2) on microalgal communities and their recolonization in the 8-day period between sprayings. The treatment had continued for at least one year. They counted the burrow density of *Nereis diversicolor* on the control and sprayed sites. No burrows were found on biocide-treated sites, whereas there were 2,280 burrows counted per m^2 at the control site. Although not studied directly, the observation suggests that *Nereis diversicolor* was excluded from the treatment site due to the toxicity of the formaldehyde or the lack of microalgal food.

5.2 Pharmaceuticals

A total of 24 results (worst-case ranked mortalities) were reported by the 10 articles that examined the effects of pharmaceuticals on polychaete species. *Hediste* (*Nereis*) *diversicolor* were examined in 54% of the studies and *Arenicola marina* were examined in 46% of the studies (Figure 5.3). ‘Severe’ mortality was reported not reported in any of the results but 21% reported ‘significant’, 4% ‘some’, 8% ‘no mortality, and the remaining 67% reported sublethal effects. However, 37% of the 67% sublethal effects were from gamete exposure experiments that examined fertilization success. The evidence is summarized below.

Da Fonseca *et al.* (2019) investigated the effects of mixtures of anticancer drugs on the polychaete *Nereis diversicolor*. The toxicity of mixtures of the drugs cisplatin (CisPt), cyclophosphamide (CP) and tamoxifen (TAM) was assessed in Mixture A: 0.1+10 +0.1 ng/l; Mixture B: 10+100+10 ng/l; Mixture C: 100 + 500 + 25 ng/l; Mixture D: 100+1000+100 ng/l, respectively. The effects on burrowing behaviour, neurotoxicity, antioxidant enzymes, biotransformation metabolism, lipid peroxidation and genotoxicity were investigated. Burrowing behaviour was assessed on day 0 before the contamination and on day 14 of the exposure. Burrowing tests were carried out over 30 minutes, with the position of the polychaete recorded every two minutes, to assess the time it took for complete burrowing to occur. The burrowing behaviour of polychaetes from both controls (Day 0 & Day 14), DMSO and from the highest tested concentration mixtures (Mixture: D) showed similar responses, that is, the polychaetes buried within 2-4 minutes. However, polychaetes exposed to Mixtures A, B and C had 33%, 13%, and 36% of polychaetes left unburied by the end of the analysis.

Fonseca *et al.* (2017) investigated the effects of mixtures of the anticancer drug Cisplatin on the polychaete *Nereis diversicolor*. The toxicity of Cisplatin was assessed by exposing the ragworms to 0.1, 10 and 100 ng/l for 14 days. Effects on burrowing, ion pump, neurotoxicity, oxidative stress, metallothionein-like proteins, biotransformation, oxidative damage, and genotoxicity were evaluated. Unexposed and exposed ragworms were subjected to a 30-minute burrowing test, where the position of each worm was recorded every two minutes to establish the time it took for the ragworm to be fully buried. Ragworms exposed to 0.1 ng/l showed similar burrowing trends as the unexposed controls, where all ragworms were burrowed within eight minutes. However, ragworms exposed to 10 and 100 ng/L showed slower burrowing rates, with 20% of individuals tested unable to burrow into the sediment by the end of the assay.

Hird *et al.* (2016) investigated the effects of fluoxetine hydrochloride a selective serotonin reuptake inhibitor (antidepressant) on the marine polychaete *Hediste diversicolor*. The polychaetes were exposed to Fluoxetine either by aqueous exposure or by sediment exposure to evaluate differences in filter-feeding and deposit-feeding marine worms. For aqueous exposure (filter feeders), the polychaetes were exposed to artificial seawater spiked with 0, 10, 100, and 500 µg/l Fluoxetine for 72 hours. In sediment exposures (deposit feeders), the polychaetes were exposed for 72 hours to sediment that had been contaminated with 0, 0.01, 0.25 and 2.5 µg/l Fluoxetine. The effects of Fluoxetine on accumulation, weight change, feeding rates, predator avoidance, metabolism, genotoxicity, and survival were evaluated. No mortalities occurred from any of the exposure treatments.

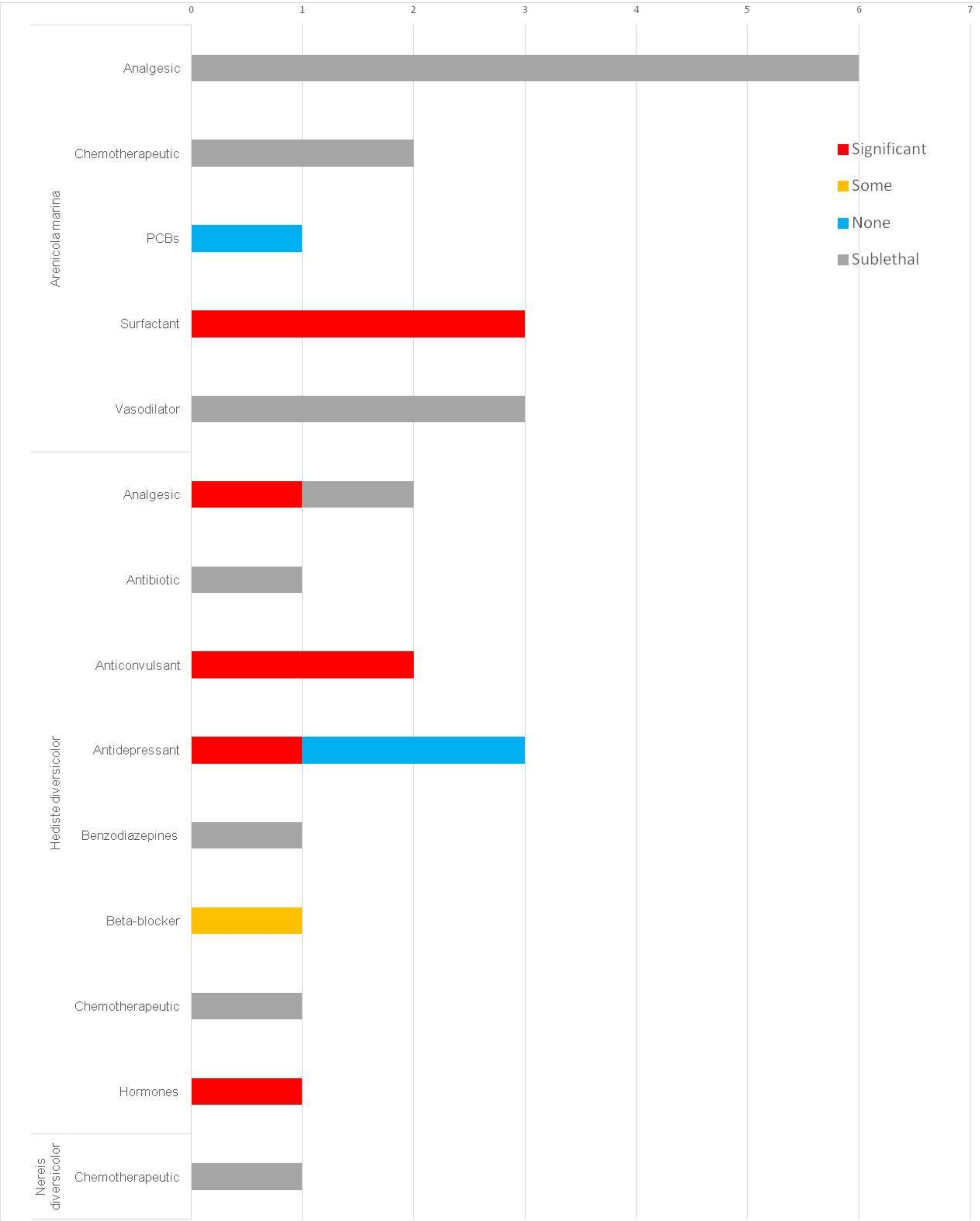

Figure 5.3. Count of worst-case ranked mortalities due to exposure to ‘Pharmaceuticals, Synthetics (other) and Polychlorinated biphenyls’ in selected polychaete species. Mortality is ranked as follows: Severe (>75%), Significant (25-75%), Some (<25%), None (no mortality reported), and Sublethal effects.

Both aqueous and sediment exposure to Fluoxetine had significant effects on the weight of the polychaetes. The aqueous exposure showed linear decreases in percentage weight change with increasing Fluoxetine, with a LOEC of 10 µg/l. No weight loss occurred in the sediment exposures. However, weight gain was significantly reduced compared to the controls, with a LOEC of 0.25 µg/g.

The feeding rate of the polychaetes was significantly affected by Fluoxetine exposure, whereby the feeding rate decreased with increasing Fluoxetine. For the filter-feeding worms (aqueous exposure), the LOEC was 10 µg/l Fluoxetine with a 68% reduction in feeding rate compared to the controls. For the deposit-feeding worms (sediment exposure), the LOEC was 0.25 µg/g with a 10% reduction in feeding rate compared to the controls. The behavioural responses of the polychaetes were influenced by Fluoxetine exposure. In the polychaetes exposed via aqueous contamination, there was an increase in the stimulus required to initiate a predator avoidance response compared to the controls with a LOEC of 500 µg/l. In the sediment-exposed polychaetes, there was a reduction in swimming speed compared to the controls with a LOEC of 0.25 µg/g Fluroxetine.

Lewis & Galloway (2009) investigated the effects of methyl methanesulfonate exposure on the fertilization rate and development of the polychaete *Arenicola marina*. Adult male polychaetes were exposed to methyl methanesulfonate at 18, 32, and 52 mg/l for 24 hours and 72 hours prior to the induction of spawning. After spawning, sperm was mixed with oocytes from unexposed females to allow for fertilization to occur. Twenty-four hours post-fertilization, oocytes/embryos were assessed for fertilization and abnormal development. Methyl methanesulfonate exposure did not affect fertilization success at all tested concentrations but abnormal development occurred at all tested concentrations.

Maranho *et al.* (2014) investigated the effects of pharmaceuticals on the polychaete *Hediste diversicolor*. Assessing the effects of pharmaceutical exposure on oxidative stress, neurotoxicity, genotoxicity, and survival of the polychaetes. The polychaete worms were exposed for 14 days to Carbamazepine, Ibuprofen, and Propranolol at 0.00005, 0.0005, 0.005, 0.05 and 0.5 µg/l and to Fluoxetine and 17α-ethynylestradiol at 0.00001, 0.0001, 0.001, 0.01 and 0.1 µg/l. Significant mortality (>25%) was observed in the Carbamazepine and Ibuprofen (0.05 and 0.5 µg/l), Fluoxetine (0.001 µg/l) and 17α-ethynylestradiol (0.01 and 0.1 µg/l) treatment groups. The survival of the polychaetes was found to be negatively correlated with concentrations of Carbamazepine and 17α-ethynylestradiol. There was no mortality reported in the control treatment.

Nogueira & Nunes (2020) investigated the effects of the antibiotic Ciprofloxacin on the biochemical and behavioural effects of the polychaete *Hediste diversicolor*. Polychaetes were exposed in acute exposures that lasted 96 hours and chronic exposures that lasted 28 days. For the acute test, the polychaetes were exposed to 0.01, 0.1, 1, 10, and 100 µg/l; and for the chronic test, the exposure concentration were 0.001, 0.01, 0.1, 1, and 10 µg/l. For the burrowing tests, the polychaetes were placed on sediment and the time it took for the individual to burrow itself was recorded. The burrowing time was affected by the acute and chronic exposures, with an increase in burrowing time compared to the controls.

Nogueira & Nunes (2021) investigated the effects of Diazepam on the biochemical and behavioural effects of the polychaete *Hediste diversicolor*. Polychaetes were exposed in acute exposures that lasted 96 hours and chronic exposures that lasted 28 days. For the acute test, the polychaetes were exposed to 0.001, 0.01, 0.1, 1, and 10 µg/l; and for the chronic test, the exposure concentrations were 0.1, 1, 10, 100, and 1000 ng/l. For the burrowing tests, the polychaetes were placed on sediment and the time it took for the individual to burrow itself was recorded. The burrowing time was affected by both acute and chronic exposures, with a decrease in burrowing time in the acute exposure and an increase in burrowing time in the chronically exposed polychaetes. A decrease in spontaneous activity was observed in exposed polychaetes compared to control organisms, under both exposure timescales. No weight changes were observed between exposed and unexposed polychaetes.

Nogueira & Nunes (2021b) investigated the effects of Paracetamol on the biochemical and behavioural effects of the polychaete *Hediste diversicolor*. Polychaetes were exposed to acute exposures that lasted 96 hours and chronic exposures that lasted 28 days. For the acute test, the polychaetes were exposed to 5, 25, 125, 625, and 3,125 µg/l; and for the chronic test, the exposure concentrations were 5, 10, 20, 40, and 80 µg/l. For the burrowing tests, the polychaetes were placed on sediment and the time it took for the individual to burrow itself was recorded. The burrowing time was affected by acute exposures, with an increase in burrowing time. An increase in spontaneous activity was observed in chronically exposed polychaetes compared to control organisms.

Pires *et al.* (2016) investigated the biochemical effects of single and combined exposure to carbamazepine and caffeine on *Hediste diversicolor*. Polychaetes were exposed to a range of concentrations of carbamazepine (0.3, 3.0, 6.0 and 9.0 µg/l) and caffeine (0.5, 3.0, and 18.0 µg/l) for 28 days. 8.3% of mortality at concentrations 0.3 and 3.0 µg/l after 28 days of exposure to carbamazepine, 25% at 6.0 µg/l and 16.7% at 18.0 µg/l. Specimens experienced 8.3% of mortality after 28 days of exposure to caffeine at 0.5 and 18.0 µg/l, and polychaetes exposed to 0.3 µg/l carbamazepine + 0.5 µg/l caffeine had 8.3% mortality. No mortality was recorded in the 3 µg/l caffeine or 3 µg/l caffeine + 6 µg/l carbamazepine treatment.

Zanuri *et al.* (2017) investigated the impacts of Diclofenac, Ibuprofen, and Sildenafil citrate (Viagra®) on the fertilization biology of spawning marine invertebrates. Effects on sperm motility and successful fertilisation were studied on the echinoderms, Asterias rubens and Psammechinus miliaris, and the polychaete worm *Arenicola marina*. Sperm motility was assessed after exposures to Diclofenac (0.01, 0.1, 1, 10, 100, 1,000 µg/l), Ibuprofen (0.01, 0.1, 1, 10, 100, 1,000 µg/l) and Sildenafil citrate (2, 10, 18, 50, 100, 1,000 µg/l) for 30, 60, 90, and 120 minutes. In the fertilization tests, sperm and oocytes were individually exposed to the test contaminants at set concentrations and times. The exposed oocytes were mixed with unexposed sperm, exposed sperm were mixed with unexposed oocytes and a combination of both exposed oocytes, and sperm were assessed for fertilization success. Sperm motility and swimming speed were reduced when exposed to >1 µg/l Diclofenac for over 90 minutes. Ibuprofen exposure significantly increased the swimming speed of the sperm when exposed to >10 µg/l for 30 minutes or longer. However, exposure to Sildenafil citrate caused no significant changes in sperm motility or swimming speed.

The results from the pre-incubation of sperm showed that Diclofenac negatively affected the fertilization success of the polychaetes with a significant reduction after 30 min of exposure at 10 µg/l. Neither Ibuprofen nor Sildenafil citrate exposure affected fertilization success significantly. The results from pre-incubation of oocytes showed that Diclofenac caused a significant decline in fertilization success at 10 µg/l. However, neither Ibuprofen nor Sildenafil citrate exposure significantly affected fertilization success. Pre-incubation of both sperm and oocytes caused significant decreases in fertilization success of the polychaetes at Diclofenac concentrations of 1 µg/l and above. Ibuprofen caused significant reductions in fertilization success when gametes were exposed to Ibuprofen at 1000 µg/l. Pre-incubation with Sildenafil citrate had no effects on fertilization success.

5.3 Synthetics (other)

The ‘synthetics (other)’ category includes a range of chemicals that do not fit into other categories conveniently. Hence, several of the chemicals included under this category only appeared in one or two studies.

Only three results were obtained from one study that reported the effects of 'Synthetics (other)' of polychaete species. The evidence is summarized below.

Conti (1987) investigated the acute toxicity of three detergents and two insecticides in the lugworm, *Arenicola marina*. Two anionic detergents: sodium dodecyl benzene sulfonate, and sodium dodecyl sulphate; one non-ionic detergent: Triton X-100; one carbamate insecticide, Carbaryl, and one organophosphate insecticide, Parathion- ethyl were used. For each toxicity test, five dose levels and one control with 10 lugworms for each dose level and control were used. The LC50 values at 48 hours for the substances were determined by the probit analysis method (Bliss, 1935). The 48-hour LC50s of the two anionic detergents were 15,200 µg/l for sodium dodecyl benzene sulphonate and 12,500 µg/l for sodium dodecylbenzenesulphonate. The 48-hour LC50 of the non-ionic detergent Triton X-100 was 15,200 µg/l and the 48-hour LC50s of the two pesticides were 2700 µg/l for parathion-ethyl and 7200 µg/l for Carbaryl.

5.4 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) were examined in only one of the articles reviewed. *Arenicola marina* was the only polychaete species examined in the study. The study reported severe mortality. The evidence is summarized below.

Casado-Martinez *et al.* (2008) investigated the suitability of lugworms (*Arenicola marina*) to study the bioaccumulation potential of Hg, PCB and PAH compounds from dredged sediments in laboratory exposures. During the study the lugworms were exposed to numerous sediment samples from several harbours that are important fishing and shipping ports near major centres of population, and, in areas mainly affected by historic mining activities but also hosting several industrial activities. The concentrations of Hg in sediments ranged between 0.05 and more than 136 mg/kg k-1 dry weight. A significant correlation between lugworm mortality and Hg concentration in sediments was observed up to 80% mortality. However, as the control sediment showed, 15–20% mortality there was the potential that mortality could have been caused by other sediment conditions and not related to chemical stress. PAH and PCB sediment concentrations were not correlated with mortality.

5.5 Sensitivity assessment – Synthetic compounds

The count of ranked 'worst –case' mortalities due to 'Synthetic compounds' are summarized in Figure 5.1 and Figure 5.2 above and Table 5.1 below. Overall, 'Severe' mortality was reported in 8.7% of the results, 'Significant' in 30.4%, 'Some' in 4%, 'no mortality in 10.8%

and sublethal effects in 45.6% of the results from the evidence reviewed. However, the toxicity and, hence, sensitivity varied between polychaete species and the chemical examined. Also, each individual chemical / species combination was only examined in a small number of studies, so that the evidence for each combination is limited.

5.5.1 *Arenicola* spp.

The effects of pesticides/biocides on *Arenicola* spp. were studied in six of the articles reviewed (Table 5.1). Garnas *et al.* (1977) reported 100% mortality in *Arenicola cristata* exposed to 1000 µg/l Kepone for 5 days. Similarly, Rubenstein (1979) reported 100% mortality in *A. cristata* exposed to 29.5 µg/l of Kepone, for 144 hours. Exposure to 2.8, 4.5, 6.6, 7.4, and 29.5 µg/l Kepone for 144 hours also reduced feeding behaviour significantly. However, Mirex and sodium pentachlorophenate reduced feeding and burrowing behaviour significantly (Schoor & Newman, 1976; Rubinstein, 1978) in *A. cristata*. Carbaryl, Ivermectin and Parathion-ethyl were reported to cause significant mortality in *A. marina* (Conti, 1987; Allen *et al.*, 2007). Ivermectin had a significant effect on the survival of the lugworms; the 10-day LC50 was 17.9 µg IVM/kg wet sediment in Test 1 and 14.8 µg IVM/kg wet sediment in Test 2 and Ivermectin reduced the mean casting rate (Allen *et al.*, 2007). Conti (1987) reported 48-hour LC50s of 2,700 µg/l for Parathion-ethyl and 7,200 µg/l for Carbaryl.

Therefore, the worst-case resistance of *Arenicola* spp. to ‘Pesticides/biocides’ is assessed as ‘None’, resilience as ‘Medium’ and sensitivity assessed as ‘Medium’. The confidence is assessed as ‘low’ due to the limited evidence and the variation in toxicity between species and the chemicals tested.

Only two papers examined the effects of ‘Pharmaceuticals’ on *Arenicola marina*. Zanuri *et al.* (2017) investigated the impacts of Diclofenac, Ibuprofen, and Sildenafil citrate (Viagra®) on the fertilization biology of spawning marine invertebrates, including *A. marina*. Sperm motility and swimming speed were reduced when exposed to >1 µg/l Diclofenac for over 90 minutes. Ibuprofen exposure significantly increased the swimming speed of the sperm when exposed to >10 µg/l for 30 minutes or longer. Diclofenac negatively affected the fertilization success of the polychaetes but neither Ibuprofen nor Sildenafil citrate exposure affected fertilization success significantly. Pre-incubation of both sperm and oocytes caused significant decreases in fertilization success of the polychaetes at Diclofenac concentrations of 1 µg/l and above. Ibuprofen caused significant reductions in fertilization success when gametes were exposed to Ibuprofen at 1000 µg/l. Pre-incubation with Sildenafil citrate had no effects on fertilization success.

Table 5.1. Summary of count of 'worst-case' ranked mortalities to 'Synthetic compounds' reported in the evidence review and resultant proposed sensitivity assessments for polychaete species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Species/Contaminant group/type/name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Arenicola cristata	**2**				**2**	**4**			
Pesticide/Biocide	**2**				**2**	**4**	**N**	**M**	**M**
Herbicide									
Sodium pentachlorophenate					1	1	H	H	NS
Organochloride									
Mirex					1	1	H	H	NS
Organohalogen									
Kepone	**2**					2	**N**	**M**	**M**
Arenicola marina	**1**	**6**			**11**	**18**			
Pesticide/Biocide		**3**				**3**	**L**	**H**	**L**
Carbomate									
Carbaryl		1				1	L	H	L
Organophosphate									
Parathion-ethyl		1				1	L	H	L
Parasiticide									
Ivermectin		1				1	L	H	L
Pharmaceuticals					**11**	**11**	H	H	NS
Analgesic									
Diclofenac					3	3	H	H	NS
Ibuprofen					3	3	H	H	NS
Chemotherapeutic									
Methyl methanesulfonate					2	2	H	H	NS
Vasodilator									
Sildenafil (Viagra)					3	3	H	H	NS
Polychlorinated biphenyls (PCBs)				**1**		**1**	**??**[6]		
Synthetics (Other)		**3**				**3**	L	H	L
Surfactant									
Sodium dodecyl sulphate		1				1	L	H	L
Sodium dodecylbenzenesulfonate		1				1	L	H	L
Triton X-100		1				1	L	H	L

[6] See text

Species/Contaminant group/type/name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Hediste diversicolor	**1**	**6**	**1**	**3**	**4**	**15**			
Pesticide/Biocide	**1**	**1**		**1**		**3**	**N**	**M**	**M**
Parasiticide									
Emamectin benzoate		1		1		2	L	H	L
Ivermectin	1					1	N	M	M
Pharmaceuticals		**5**	**1**	**2**	**4**	**12**	**L**	**H**	**L**
Analgesic									
Ibuprofen		1				1	L	H	L
Paracetamol					1	1	H	H	NS
Antibiotic									
Ciprofloxacin					1	1	H	H	NS
Anticonvulsant									
Carbamazepine (CBZ)		2				2	L	H	L
Antidepressant									
Fluoxetine		1		2		3	L	H	L
Benzodiazepines									
Diazepam					1	1	H	H	NS
Beta-blocker									
Propranolol			1			1	M	H	L
Chemotherapeutic									
Cisplatin					1	1	H	H	NS
Hormones									
17α-ethynylestradiol		1				1	L	H	L
Nereis diversicolor		**2**	**1**	**2**	**1**	**6**			
Pesticide/Biocide		**2**	**1**	**2**		**5**	L	H	L
Insecticide									
Carbaryl				1		1	H	H	NS
Organophosphate									
Malathion			1			1	M	H	L
Parathion		1				1	L	H	L
Phosalone				1		1	H	H	NS
Parasiticide									
Ivermectin		1				1	L	H	L
Pharmaceuticals					**1**	**1**			
Chemotherapeutic									
Cisplatin, Cyclophosphamide, Tamoxifen					1	1	H	H	NS
Eteone longa					**1**	**1**			
Pesticide/Biocide					**1**	**1**	H	H	NS
Carbomate									
Carbaryl					1	1	H	H	NS

Species/Contaminant group/type/name	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
***Nephtys* sp.**					**1**	**1**			
Pesticide/Biocide					**1**	**1**	H	H	NS
Organohalogen									
Aldrin					1	1	H	H	NS
Streblospio benedicti					**1**	**1**			
Pesticide/Biocide					**1**	**1**	H	H	NS
Carbomate									
Carbaryl					1	1	H	H	NS
Overall total	**4**	**14**	**2**	**5**	**21**	**46**			

Lewis & Galloway (2009) reported that methyl methanesulfonate exposure did not affect fertilization success at all tested concentrations but abnormal development occurred at all tested concentrations (18, 32, and 52 mg/l for 24 hours and 72 hours prior to the induction of spawning) in polychaete *Arenicola marina*.

Therefore, the sensitivity of *Arenicola marina* to 'Pharmaceuticals' is assessed as 'Not sensitive', but with 'Low' confidence due to the limited evidence. However, reported effects on larval development may have long-term effects on recruitment and population dynamics.

Casado-Martinez *et al.*, 2008 examined the effects of Hg, PCB and PAH contamination on *Arenicola marina* but PAH and PCB sediment concentrations were not correlated with mortality. Therefore, **no assessment of its sensitivity to PCBs is attempted**.

Conti (1987) examined the effects of surfactants on *A. marina*. The 48-hour LC50s of the two anionic detergents were 15,200 µg/l for sodium dodecyl benzene sulphonate and 12,500 µg/l for sodium dodecylbenzenesulphonate. The 48-hour LC50 of the non-ionic detergent Triton X-100 was 15,200 µg/l. **Therefore, the worst-case resistance of *Arenicola* spp. to surfactants (detergents) is assessed as 'Low', resilience as 'High' and sensitivity assessed as 'Low'.** The confidence is assessed as 'low' due to the limited evidence.

5.5.2 *Hediste* spp. and *Nereis* spp.

Seven articles examined the effects of 'Pesticides/biocides' on *Hediste* (*Nereis*) *diversicolor*. For example, Collier & Pinn (1998) reported that *Nereis diversicolor* was the most sensitive of the species in their experiment, as 8.0 mg/m^2 Ivermectin caused 100% mortality within 14 days. Mayor *et al.* (2008) reported that the sea-lice insecticide Emamectin benzoate had significant effects on the survival of *Hediste diversicolor* with an LC50 value of 1,367.71 µg/kg (wet sediment). Scaps *et al.* (1997) reported that by day 21, 20% of *Nereis diversicolor* exposed to 10^{-6} and 10^{-8} M Malathion died, and 33.5 and 62.5% mortality occurred in the 10^{-8} and 10^{-6} M parathion-ethyl treatments. Only sublethal effects were reported in the other pesticides reviewed. Underwood & Paterson (1993) reported that *Nereis diversicolor* was absent from areas of sediment treated with formaldehyde. Although not studied directly, the observation suggests that *Nereis diversicolor* was excluded from the treatment site due to the toxicity of the formaldehyde or the lack of microalgal food.

Therefore, the worst-case resistance of *Nereis* spp. and *Hediste* spp. to 'Pesticides/biocides' is assessed as 'None', resilience as 'Medium' and sensitivity assessed as 'Medium'. The confidence is assessed as 'low' due to the limited evidence and the variation in toxicity between species and chemicals tested.

Eight articles examined the effects of several 'Pharmaceuticals' on *Hediste* (*Nereis*) *diversicolor*. For example, Maranho *et al.* (2014) reported that the survival of the *Hediste diversicolor* was negatively correlated with concentrations of Carbamazepine and 17α-ethynylestradiol. 'Significant' mortality (>25%) was observed in the Carbamazepine (0.05 and 0.5 µg/l), Fluoxetine (0.001 µg/l) and 17α-ethynylestradiol (0.01 and 0.1 µg/l) treatment groups. Pires *et al.* (2016) reported that *Hediste diversicolor* experienced 8.3% mortality at concentrations 0.3 and 3.0 µg/l after 28 days of exposure to carbamazepine, 25% mortality at 6.0 µg/l and 16.7% mortality at 18.0 µg/l after 28 days of exposure carbamazepine. 'Some' mortality occurred after exposure to the beta-blocker Propranolol (Maranho *et al.*, 2014). However, the other studies reported only sublethal effects, including a reduction in burrowing activity.

Therefore, the worst-case resistance of *Nereis* spp. and *Hediste* spp. to 'Pharmaceuticals is assessed as 'Low', resilience as 'High' and sensitivity assessed as 'Low'. The confidence is assessed as 'low' due to the limited evidence and the variation in toxicity between the chemicals studied.

5.5.3 *Nephtys* spp., *Eteone longa*, and *Streblospio benedicti*

Nephtys spp., *Eteone longa*, and *Streblospio benedicti* were examined in only two of the articles reviewed. In all cases, only sublethal effects were reported. For example, Dumbauld *et al.* (2001) observed the effects of the application of the pesticide Carbaryl on the estuarine benthic community in oyster culture sites but did not detect trends or significant differences in the abundance of the polychaetes *Streblospio benedicti* and *Eteone longa*. Similarly, Scanes *et al.* (1993) reported no significant changes in polychaete abundance (inc. *Nephtys* spp.) after an accidental spill of the pesticide Aldrin on an estuarine beach in New South Wales, Australia.

Therefore, the **sensitivity of *Nephtys* spp., *Eteone longa*, and *Streblospio benedicti* to ‘Synthetic compounds’ is assessed as ‘Not sensitive. The confidence is ‘Low’ due to the lack of evidence.**

6 Other substances and sensitivity assessment

'Other substances' include a range of chemicals that do not fit into the other categories of contaminant. Neither do they group conveniently. Therefore, the results of individual chemicals are tabulated in Table 6.1 and the evidence summarized below.

Table 6.1. Summary of count of 'worst-case' ranked mortalities to 'Other substances' reported in the evidence review and resultant proposed sensitivity assessments for polychaete species (N= None, VL= Very low, L= Low, M= Medium, High = High, and NS= Not sensitive).

Species Name	Group/Type	Chemical name	Severe	Significant	Some	Sublethal	Total	Resistance	Resilience	Sensitivity
Arenicola marina										
	Inorganics									
		Single Walled Carbon Nanotubes				**1**	1	H	H	NS
Hediste diversicolor										
	Inorganics									
		Graphene		**1**			1	L	H	L
		Multi-walled carbon nanotubes			**1**		1	M	H	L
	Natural product									
		Caffeine			**1**		1	M	H	L
	Other									
		Antifouling paint particles	**1**				1	N	M	M
Total			**1**	**1**	**2**		**4**			
Overall total			**1**	**1**	**2**	**1**	**5**			

6.1 Inorganic chemicals

Galloway *et al.* (2010) investigated the sublethal toxicity of nano-titanium dioxide and carbon nanotubes on the marine polychaete *Arenicola marina*. The lugworms were exposed through natural sediments to a 10-day OECD/ASTM 1990 acute toxicity test. Sediment was prepared with either single-walled carbon nanotubes (0.003–0.03 g/kg), nano-titanium dioxide (1–3 g/kg), or seawater alone. The lugworms were fed every other day and feeding behaviour was monitored every 48 hours. Casts were collected, dried overnight and weighed, with seawater renewals every 48 hours following cast collection. After 10 days of exposure, the lugworms were removed from the exposure sediment and the lugworm's ability to re-bury into clean sediment was assessed following the OECD/ICES A. marine burrowing bioassay. Sediment exposure to single-walled carbon nanotubes or nano-titanium dioxide had no effects on the burrowing behaviour of the lugworm. During the exposure period, single-walled carbon nanotubes had no effects on the feeding behaviour of the lugworms. However, nano-titanium dioxide exposure caused a significant impact on feeding behaviour with reductions in casting rate at 2 g/kg nano-titanium dioxide.

Therefore, *Arenicola marina* is probably 'Not sensitive' to single-walled carbon nanotubes (at 0.003–0.03 g/kg). However, confidence in the assessment is 'low' due to the lack of evidence.

De Marchi *et al.* (2017) investigated the effects of different multi-walled carbon nanotubes (MWCNTs) (at 0.01; 0.10 and 1.00 mg/l) on *Diopatra neapolitana* and *Hediste diversicolor*. The effects on physiological and biochemical performance were assessed after 28 days of exposure. The respiration rate of *Hediste diversicolor* was measured after 28 days of exposure. Exposure to 0.01 mg/l MWCNTs increased the respiration rate compared to the controls. However, respiration rates significantly decreased at 0.1 mg/L MWCNTs compared to the control, but respiration rate increased at the highest tested concentration (1.00 mg/L) of MWCNTs. Mortality of *H. diversicolor* individuals exposed to 0.01, 0.10 and 1.00 mg/l was 11% at each of the tested concentrations. In the control treatment, there was 100% survival recorded after bioassay.

Pires *et al.* (2022) investigated the effects of graphene oxide (GO) nanosheets on the behavioural, physiological, and biochemical responses of *Hediste diversicolor*. Polychaetes were exposed to a range of concentrations of graphene oxide nanosheets (10, 100, 1000, and 10,000 µg/l) for 28 days. The study assessed the effects on the behaviour, feeding activity, mucus production, regenerative capacity, antioxidant status, biochemical damage, and metabolism. Body regeneration was significantly influenced by GO exposure, with all individuals exposed to GO exhibiting reductions in the number of regenerated segments when compared to the controls. Feeding activity was influenced by GO exposure with increased feeding times for exposed individuals. The segregation of mucus was significantly higher in individuals exposed to GO when compared to the controls. Burrowing rates of polychaetes exposed to GO were significantly slower than those in the control, with 20-35% of individuals in the highest tested concentrations unable to burrow by the end of the 30-minute assay. Mortality was around 40% in the GO-exposed treatments, with 30% mortality in the 10 and 100 µg/l treatments, but only 5% in the highest tested concentration of 10,000 µg/l. No mortality occurred in the control.

Therefore, ***Hediste diversicolor* probably has a resistance of 'Low' to graphene oxide nanosheets and 'Medium' resistance to multi-walled carbon nanotubes. Hence, resilience is probably 'High' and sensitivity is assessed as 'Low'** but with 'Low' confidence.

6.2 Natural products

Pires *et al.* (2016) investigated the biochemical effects of single and combined exposure to Carbamazepine and caffeine on *Hediste diversicolor*. Polychaetes were exposed to a range of concentrations of carbamazepine (0.3, 3.0, 6.0 and 9.0 µg/l) and caffeine (0.5, 3.0, and 18.0 µg/l) for 28 days. 8.3% of mortality at concentrations 0.3 and 3.0 µg/l after 28 days of exposure to carbamazepine, 25% at 6.0 µg/l and 16.7% at 18.0 µg/l. Specimens experienced 8.3% of mortality after 28 days of exposure to caffeine at 0.5 and 18.0 µg/l, and polychaetes exposed to 0.3 µg/l carbamazepine + 0.5 µg/l caffeine had 8.3% mortality. No mortality was recorded in the 3 µg/l caffeine or 3 µg/l caffeine + 6 µg/l carbamazepine treatment.

The above evidence **suggests that *Hediste diversicolor* has a 'Low' sensitivity to caffeine** exposure but confidence in the assessment is 'Low' due to the lack of evidence.

6.3 Other

Muller-Karanassos *et al.* (2021) investigated the effects of environmental concentrations of antifouling paint particles on sediment-dwelling invertebrates. Adult ragworms and cockles were exposed to three types of antifouling paint particles (APP), two biocidal ('historic' and 'modern') and one biocide-free ('silicone'). Two laboratory-based 18-day and 5-day exposure experiments were carried out. The APPs ranged in particle size and included varying concentrations of Cu, Sn, Pb, Hg, and Zn. Trial experiments carried out using the maximum environmental APP concentration (18.8 g/l) caused 100% mortality of all ragworms and cockles in the modern treatment within 6 days. In the 18-day exposure, antifouling paint particle concentrations were 4.2 g/l for the historic biocidal treatment; 3.0 g/l for the modern biocidal treatment; and 2.1 g/l or the non-biocidal silicone treatment. The burrowing rate of the ragworms was reduced by 29% in the modern biocidal treatment. However, there were no significant differences between treatments. Ragworms decreased in weight and feeding rates significantly, but significant differences were only seen between the modern biocidal treatment and the control. Modern biocidal antifouling paint particles were used at concentrations ranging from 0 to 30 g/l (ragworms) and 0 to 6 g/l (cockles) to estimate the 5-day LC50 exposure. The 5-day LC50 values were 19.9 g/l for the ragworms and 2.3 g/l for cockles. The 5-day EC50 values were 14.6 g/l for the ragworms and 1.4 g/l for cockles.

The evidence Muller-Karanassos *et al.* (2021) suggests that antifouling paint particles remain toxic in the environment. **Therefore, the resistance of *Hediste diversicolor* to APPs is assessed as 'None'. Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'** but confidence in the assessment is 'Low' due to the lack of evidence.

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7 Conclusions

This report presents the finding of a time limited Rapid Evidence Assessment (REA) of the effects of contaminants on selected polychaetes. The literature review focused on *Ampharete* spp., *Aphelochaeta* spp., *Arenicola* spp., *Hediste* spp., *Nereis* spp., *Nephtys* spp., *Pygospio* spp., *Eteone* spp., *Lanice* spp., *Streblospio shrubsolii* and the oligochaete *Tubificoides* spp.. The key findings are summarized below.

- 'Transitional metals' was the most studied contaminant group and contributed 52.8% of the results in the evidence review followed by 'Pharmaceuticals' (12.4%), 'Pesticides/biocides' (9.8%), petrochemical hydrocarbons (8.5%), and the remaining groups of contaminants contributed less than 16.5% of the results

- *Arenicola* spp. and *Nereis* spp. were the most studied genera and each contributed ca 33% of the results in the evidence reviewed, closely followed *Hediste* spp., which contributed 28% of the results. The remaining genera each contributed less than 1.5% of the results in the evidence reviewed.

- *Lanice* spp. and *Aphelochaeta* spp. were mentioned in only two studies of organic/nutrient enrichment from sewage outfalls (Conlan *et al.*, 2004; Bergayou *et al.*, 2019) which are outside the scope of this study.

- *Nephtys* spp., *Eteone* spp., *Tubificoides* spp. and *Streblospio* spp. were examined in a small number of the articles reviewed.

- 'Transitional metals' were the most toxic contaminant group studied, in terms of mortality but also the most studied. However, toxicity varied between the different metals, species, and environmental conditions

- 'Pesticides/biocides' were reported to be the next most toxic, followed by 'petroleum hydrocarbons' and 'Pharmaceuticals'. However, the toxicity of pesticides/biocides varied depending on the chemical and species studied.

- The ability of polychaetes (e.g., Nereis) to detoxify and/or adapt to transitional metal contamination was not included in the sensitivity assessments directly. It was assumed that adaptation required long-term exposure, while the sensitivity assessments represent their likely (or possible) response of polychaete species to a new or recent event, e.g., a spill.

- The volume of evidence (in terms of number of relevant articles found) was surprisingly low, except for the well-studied genera *Arenicola, Hediste,* and *Nereis*. And in most cases the effects of any one contaminant was only examined in one to four articles. Hence, most of the sensitivity assessments included in the report are made with 'Low' confidence due to the limited evidence.

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8 Bibliography

8.1 Articles included in the evidence review

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9 Appendix 1

The evidence extracted (or mapped) is limited to fields likely to be relevant to sensitivity assessment or to categorise the 'level of effect' recorded in each article. The extensive systematic map suggested by Randall *et al.* (2015) was felt to be too onerous.

The field names and standard terms used within the 'Evidence summaries' were developed during Phase 2 and 3, based on terms used by the US EPA ECOTOX database or MarLIN glossary, or adapted from the literature review, wherever possible or relevant. Not reported (NR) is used wherever the relevant data/evidence is not reported or specified in the evidence. The field names and relevant standard terms follow.

Short citation

Standard short form of citation for article/paper/book/ report etc.

Study type

Outline of the type of study adapted from ECOTOX definitions:

Term	Definition
Field (obs.)	Observation in the field e.g., effect of spills, physical disturbance
Field (expt.)	Field based study, e.g., in situ mesocosm, field based experimental design exposed and control plots/quadrats/transects
Laboratory	Experimental or observational study conducted under laboratory conditions
Mesocosm	Experimental or laboratory studies conducted within mesocosms either based in the laboratory or the field
Review	Review article (paper/report). Reviews used as sources of evidence and only novel data in reviews included, originals articles examined for detail
Survey	Survey of multiple site presence/absence/abundance etc. of chemical or species

Note –chemical analysis requires access to a laboratory but is not included within the study type.

Chemical names and groups

'Contaminants group,' 'contaminant type,' 'contaminant name' and 'CAS number' from the agreed 'Contaminant Chemicals Groups' March 2022' spreadsheet. Two versions of 'contaminant name' are listed:

- 'Contaminant name' reported by the article cited, and
- 'Contaminant synonym' used by ECOTOX or others, if available and different from 'contaminant name'.

Species name

The name of the species studied as reported in the original article. Relevant synonyms, based on WoRMS[7], are used in the report text.

Life stage studied

Terms defined in MarLIN glossary

- Adult
- Juvenile
- Larvae
- Embryo
- Egg
- Sporophyte
- Gametophyte
- Multiple

Exposure concentration

The experimental concentrations the samples were exposed to, where available, and expressed in reported units and µg/l where possible.

Exposure type

Definitions of the type or route of exposure to the contaminant, adapted from ECOTOX.

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[7] WoRMS – World Register of Marine Species - https://www.marinespecies.org/index.php

Term	Definition
Environmental	Field and incidental exposures, includes via the water column or sediment
Environmental (sediment)	Optional where sediment concentration are paramount (e.g., sedimentary communities)
Flow-through	Continuous or frequent flow through test chamber with no recycling
Food	Introduced via food
Lentic	Static water without measurable flow e.g., lakes, ponds, lagoons
Pulse	Intermittent or fluctuating dosing
Renewal	Without continuous flow of solution, but with occasional renewal of test solutions after prolonged periods, e.g., 24 hours
Spill	Incidental spills
Static	Toxicity tests with aquatic organisms in which no flow of test solution occurs; solutions may remain unchanged throughout the duration of the test.
Tidal	Affected by tides

Study duration

The length of the study and reported by article in hours, days, months, or years etc.

Exposure Duration (ECOTOX definition)

The Exposure Duration is the time of actual exposure to the chemical and is expressed as 'days'. In cases where the observation time is the only duration reported, it is assumed that the Exposure Duration is equivalent to the longest observation time (field: Observed Duration).

For most field studies the 'Exposure' and 'Study Duration' are identical because it is difficult to determine when the exposure ends. For lab studies the 'Exposure' and 'Study Duration' may be different, such as when effect measurements were reported from a post-exposure period. For lab studies with injection, topical, or dietary (e.g., intraperitoneally or by gavage) exposure, 'Exposure and Study Duration' are typically the same.

For a fluctuating or intermittent dosing experiment, the total exposure time is recorded. In some instances, a biological, or qualitative, time is used, such as an exposure time reported as "until hatch", "growing season" or "after the nth egg has been laid".

Effect group (definitions from ECOTOX)

Term	Definition
Accumulation	Measurements and endpoints that characterize the process by which chemicals are taken into and stored in plants or animals; includes lethal body burden
Behaviour/Avoidance,	Activity of an organism represented by three effect groups - avoidance, general behaviour, and feeding behaviour
Biochemical (inc. enzyme(s), hormone(s))	Measurement of biotransformation or metabolism of chemical compounds, modes of toxic action, and biochemical responses in plants and animals; includes three effect groups - biochemical, enzyme and hormone effects
Cellular/ Histology/ Genetic	Measurements and endpoints regarding changes in structure and chemical composition of cells and tissues of plants or animals as related to their functions; includes three effect groups -cellular, genetic, and histological effects
Ecosystem process	Measurements and endpoints to track the effects of toxicants on ecosystem processes; includes microbial processes
Growth/ Development/ Morphology	Category encompasses measures of weight and length, and includes effects on development, growth, and morphology
Mortality	Measurements and endpoints where the cause of death is by direct action of the chemical
Multiple	Measurements related to multiple or undefined effect.
No Effect	The author reported an end point but not a specific effect
Physiology/ Immunological/ Injury/ Intoxication	Measurements and endpoints regarding basic activity in cells and tissues of plants or animals; includes four effect groups - injury, immunity, intoxication, and general physiological response
Population	Measurements and endpoints relating to a group of organisms or plants of the same species occupying the same area at a given time
Reproduction	Measurements and endpoints to track the effect of toxicants on the reproductive cycle; includes behavioural and physiological measurements

Effect measurement

A description of the effect measured. These are likely to vary between different taxonomic groups. The ECOTOX database includes many more categories than listed below for some of the ‘effect groups;’ the numbers are given in brackets. Examples of standard ‘effect measurement’ terms, organized by ‘effect group,’ include:

- Accumulation
  - Body burden
  - BCF
- Behaviour/Avoidance
  - Chemical avoidance
  - Substratum avoidance
- Biochemical (ECOTOX =1,641 entries)
  - Acyl-CoA oxidase activity
  - Acetylcholinesterase (AchE) activity
  - Acid phosphatase
  - Catalase (CAT)
  - Cytochrome P450 activity
  - Gamma-Glutamyl Transpeptidase
  - Glutathione disulphide
  - Glutathione peroxidase (GPX),
  - Glutathione reductase (GR),
  - Heat shock proteins
  - Lactate dehydrogenase
  - Lipid peroxidation,
  - Metallothioniens
  - MFO (BPH, CYP-dependent monoxygenase)
  - Multixenotoxicity resistance
  - NADPH-Neo tetrazolium Reductase activity
  - NF-E2-related factor 2 (Nrf2),
  - Superoxide dismutase (SOD)
- Cellular (ECOTOX has 143 entries)
  - DNA damage/Micronuclei/Adduct formation
  - Genotoxicity
  - Haemocyte counts population

  - Phagocytosis
  - Lysosomal membrane stability
  - Ovarian and spermatic follicles
  - Transmembrane sodium energy gradient
  - Transcriptomics
- Ecosystem processes
  - General
  - Reduced/Increased productivity (primary/secondary)
  - Community
- Growth/Development/Morphology
  - Abnormal development/larvae
  - Growth rate
  - Leaf/shoot/rhizome/root elongation
  - Leaf shape/morphology
  - Mortality (adult/larval)
  - Adult survival
  - Larval survival
- Physiology/Immunological/Injury/Intoxication
  - Byssal thread production
  - Clearance/filtration rate
  - Excretion rate
  - Larval swimming velocity/ability
  - Respiration rate
  - Condition indices
  - Photosynthetic efficiency
  - PSII function/damage
  - Scope for growth (SFG)
  - Valve gape
  - Population
  - Abundance/biomass
  - Condition
  - Cover/canopy
  - Distribution/extent
  - Diversity
  - Population decline (general)

- Reproduction
  - Fecundity
  - Gametogenesis reduction
  - Gonad index
  - Fertilization success/failure
  - Recruitment success
  - Settlement
  - Sexual maturity (rate/age)
  - Sex ratios
  - Imposex

Response site

The part (or type) of the organism where the effect (response) is measured (or observed). ECOTOX has 594 entries, which vary between taxonomic groups. We should expect to add terms as we tackle more taxonomic groups but use ECOTOX definitions where possible. For example:

- Community
- Digestive gland
- Embryo
- Gametes (oocytes and sperm)
- Gonad
- Haemocytes
- Larva
- Leaf/shoot
- Lysosomes
- Muscle tissue
- Rhizomes/roots
- Population
- Seedling
- Soft tissues
- Whole organism (assumes adult)

End points

List of observed end points reported by the articles examined, used for consistency with ECOTOX data, but also includes population level effects due to environmental exposure, spills etc. For example:

- BCFD - Bioconcentration factor calculated using dry weight tissue concentration
- ECXX– Effect concentration at XX percentile
- ICXX - Inhibition concentration at XX percentile
- IDXX - Inhibition dose at XX percentile
- LCXX– Lethal concentration at XX percentile
- LDXX – Lethal dose at XX percentile
- LTXX – Lethal time at XX percentile
- LOEC/L – Lowest Observable-Effect-Concentration/Level: lowest dose (concentration) producing effects that were significantly different (as reported by authors) from responses of controls (LOEAL/LOEC)
- NOEC/L – No Observable-Effect-Concentration/Level: highest dose (concentration) producing effects not significantly different from responses of controls according to author's reported statistical test (NOEAL/NOEC)
- Mortality (e.g., after spills)
- NR-LETH – 100% Mortality
- NR-ZERO – 0% Mortality
- Population loss
- Population decline
- Recruitment failure

Endpoint concentrations

ECOTOX provides a single concentration or range (with or without confidence intervals) for each Endpoint. ECOTOX lists the confidence intervals as a range (min, max). In the 'Evidence summary' different End point concentrations (or ranges) are listed separately. Lethal (100%) is included where papers give a concentration resulting in 100% mortality, which is one endpoint recorded by ECOTOX.

Concentrations are expressed as mg/l (ECOTOX) and/or µg/l.

Mortality (%) reported

The percentage mortality reported in the articles examined, where available.

Ranked mortality

The mortality reported in the articles examined is ‘ranked’ according to the MarESA resistance scale. For example:

Ranked mortality	Resistance
Severe (>75%)	None
Significant (25-75%)	Low
Some (<25%)	Medium
None (reported)	High
Sublethal	High
Unspecified	Unspecified

Unspecified = mortality is reported but not quantified or no detail provided

Quality/Applicability of Evidence – based on MarESA scales

Summary of evidence

The relevant evidence from the articles is summarized in narrative form, using the standard MarESA format description of evidence.

‘Worst-case’ ranked mortality

The reported ‘end points’ and evidence from each article is expressed as a ‘worst-case’ ranked mortality for each contaminant examined in each article. For example, where the specimens are exposed to a range of concentrations of one chemical and several ‘end points’ (e.g., EC50, LC50) determined, the ‘worst-case’ or greatest mortality is reported.

Please note, many papers examined several different combinations of contaminant type and species. Therefore, the ‘worst case’ mortality is recorded for each unique species vs. contaminant combination within each paper but not for every experimental permutation. For example, if a paper studied three metals and one herbicide, then we would report the four ‘worst case’ mortalities rather than every mortality or effect from every concentration tested. However, if the papers examined the same combination on three distinct species (e.g., in seagrasses) then we would record twelve separate ‘worst-case’ mortalities.

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