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Sensitivity Assessment of Contaminant Pressures - Selected Molluscs - Evidence review.

DATE: May 2023

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Recommended citation:

Williams, E. & Tyler-Walters, H., 2023. Sensitivity Assessment of Contaminant Pressures – Selected Molluscs – Evidence Review. MarLIN (Marine Life Information Network), Marine Biological Association of the UK, Plymouth, pp. 79. Available from https://www.marlin.ac.uk/publications

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1 Introduction

An evidence review of the effects of contaminants on selected species of molluscs 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 'Mollusc 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), and
- 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 'Mollusc 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 *Cerastoderma* spp., *Macoma* spp. (plus the syn. *Limecola balthica*) and *Scrobicularia* spp.. Other genera were excluded to keep the literature review manageable.

The initial searches (12th December 2022) resulted in ca 2,375 hits of which 1,408 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 82 articles[3], which were taken forward for detailed review. However, 19 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 the selected 'molluscs'.

Review stage	No. articles identified/retained	No. articles rejected/removed
Web of Science	2,298	
ECOTOX database	77	
Duplicates removed	967	1,408
Screening	104	781
Taken forward*	82	
Not accessible	19	

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

A total of 264 results were obtained from 82 articles that studied the effect of contaminants on the selected mollusc species. Overall, the articles reviewed reported mortality ('Severe' to 'Some') in 78% of results (worst-case ranked mortalities), no mortality ('none') in 8.7% of results, and sublethal effects in 13% of results. The largest proportion of sublethal effects was reported in studies of the effects of 'Pharmaceuticals' (Figure 2.1).

[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.

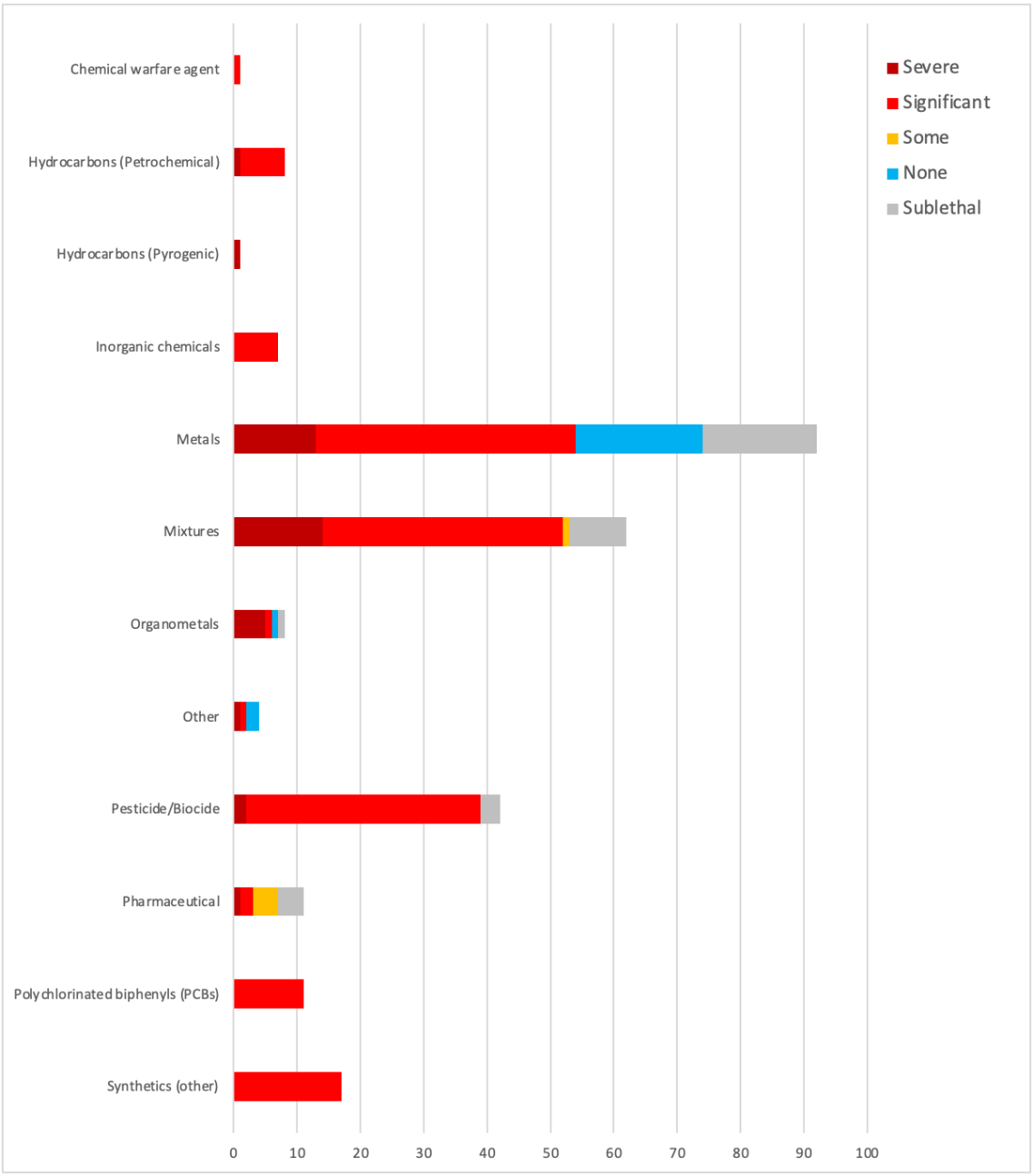

Figure 2.1. Count of worst-case ranked mortalities due to exposure to contaminants in selected molluscs. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects.

'Transitional metals' was the most studied contaminant group and contributed *35%* of the results in the review. 'Hydrocarbons (Petrochemical)' dispersant and oil mixtures contributed 26% of the results and 'Pesticides/biocides' 15% of the results from the evidence review.

Cerastoderma spp. was examined in 24 (29%) of the studies reviewed and contributed 62% of the results in the review to the widest range of contaminant types (Figure 2.2). In comparison, *Scrobicularia* spp. was examined in 32 (39%) of the studies and contributed

19% of the results and *Macoma* spp. was examined in 34 (41%) of the studies but contributed 18% of the results (Figure 2.2).

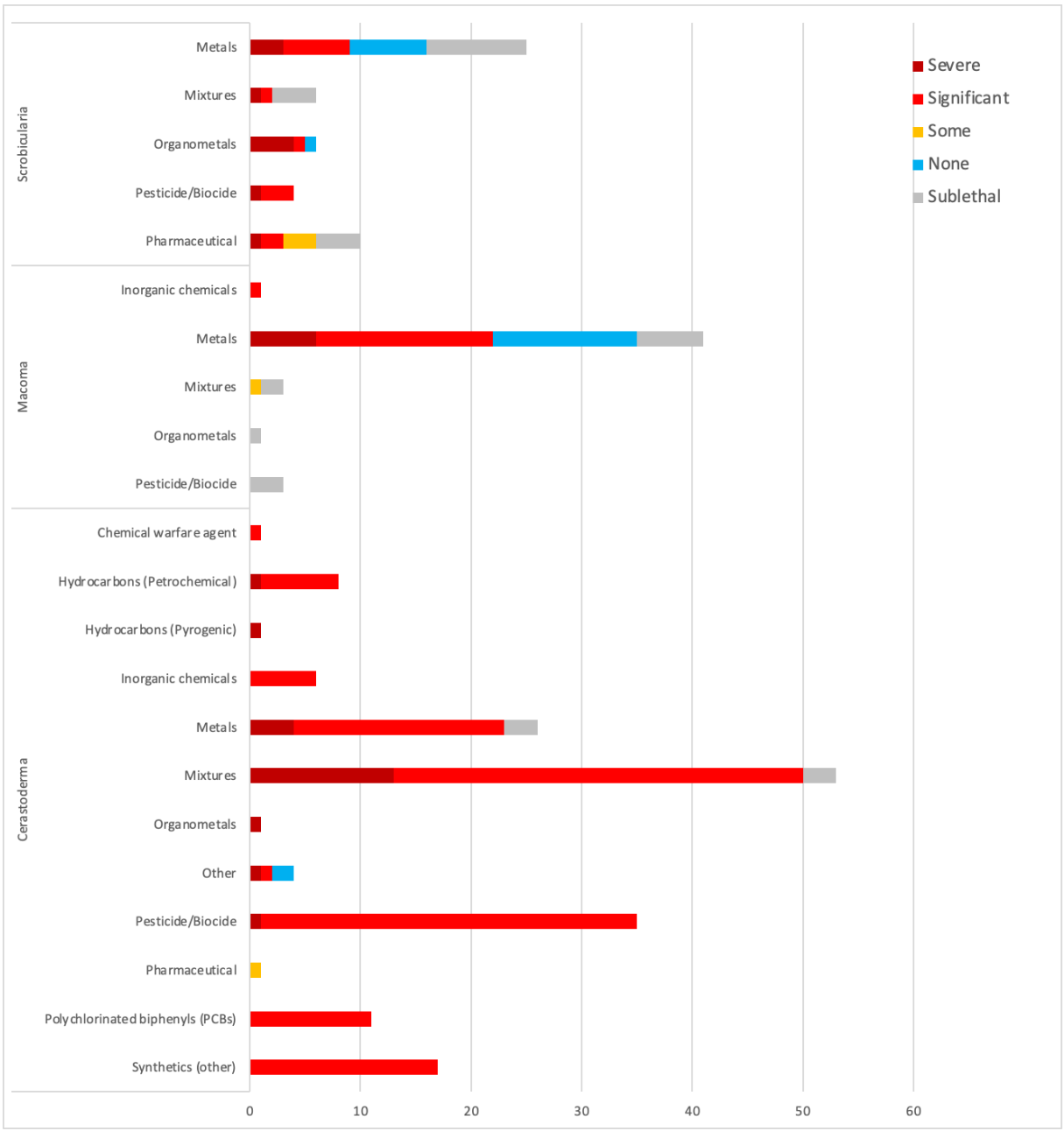

Figure 2.2. Count of worst-case ranked mortalities due to exposure to contaminants in selected molluscs by taxonomic genera. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects.

3 Hydrocarbons and PAHs

At total of 57 results were obtained from eight articles that studied the effect of hydrocarbons, PAHs, and dispersants on molluscs, of which 2% examined polyaromatic hydrocarbons, 12% examined the effects of petrochemicals including phenols, aromatic hydrocarbons, and complex hydrocarbons and 86% examined the effects of mixtures such as dispersants and oil emulsions (Figure 3.1). The majority (98%) of the obtained results examined the effects of hydrocarbons, PAHs, and dispersants on *Cerastoderma* spp., with only one result for *Macoma balthica* and no results for *Scrobicularia* spp.

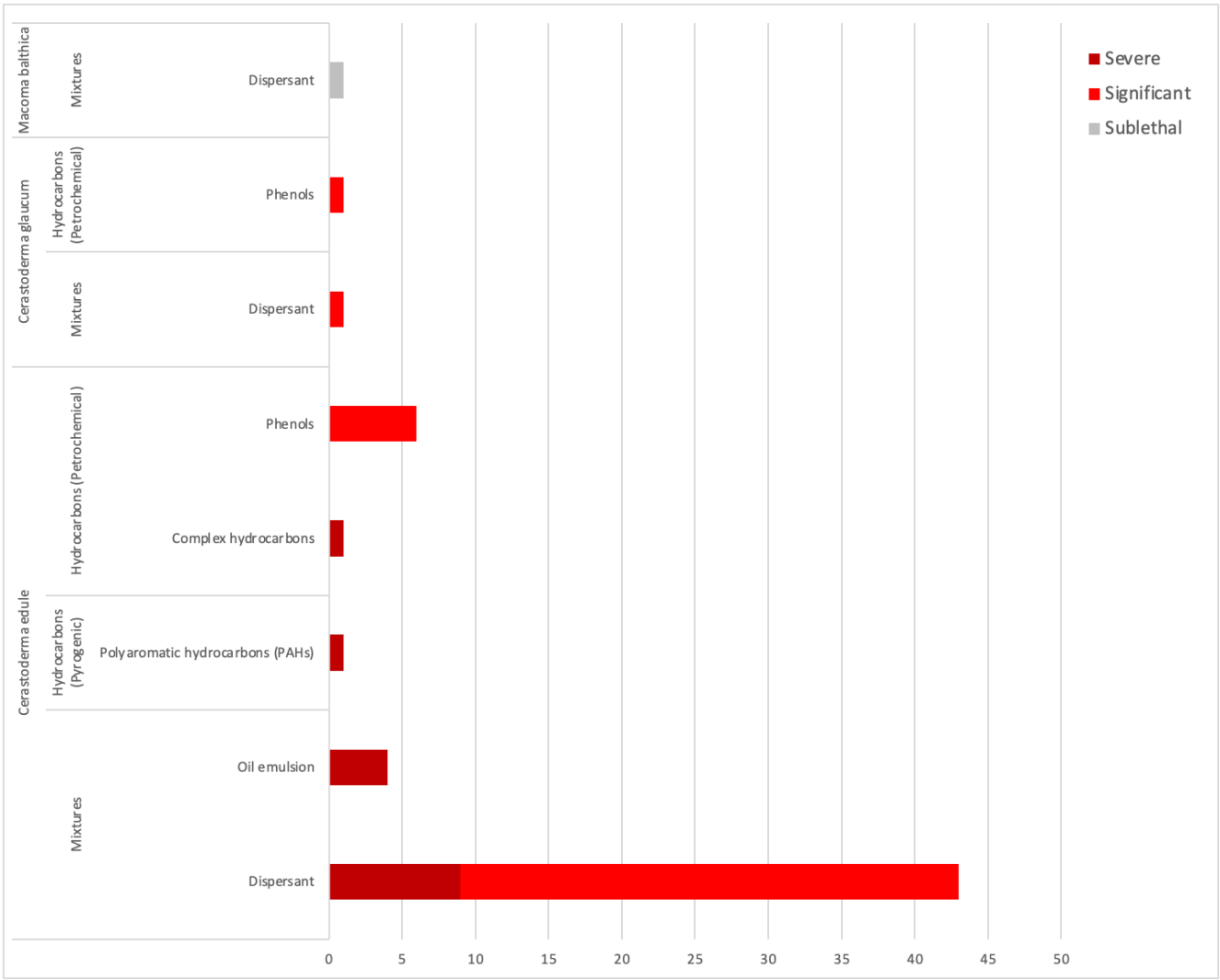

Figure 3.1. Count of worst-case ranked mortalities due to exposure to hydrocarbons, PAHs, dispersants, and oil emulsion in selected mollusc species. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

3.1 Petroleum hydrocarbons – oils and dispersed oils

Only one of the articles examined the effects of petroleum (e.g., crude oil and fuel/bunker oils), and dispersed oils on the selected molluscs reviewed.

Swedmark *et al.* (1973) investigated the toxicities of nine oil dispersants, three oil emulsions with Corexit and a dispersion of Oman crude oil on eight marine organisms. The test organisms were *Gadus morhua* (cod), *Pleuronectes flesus* (flounder), *Pecten opercularis* (scallop), *Cerastoderma* (as *Cardium*) *edule* (cockle), *Mytilus edulis* (mussel), *Leander adspersus* var. *fabricii* Rathke (prawn), *Hyas araneus* (spider crab) and *Eupagurus bernhardus* (hermit crab). The study was conducted in continuous flow aquarium systems at 96-hour exposures followed by a recovery period of 48 hours in clean seawater. The cockles and scallops were more sensitive to the dispersants than mussels. Fina-Sol SC and BP 1100 X were the most toxic to the cockles and scallops with 96-hour LC50s of <40 ppm. BP 1100 X, Corexit 8666 and Fina-Sol OSR-2 had the least effect on cockles, scallops, and mussels and did not cause 50% mortality within 96 hours (96-hour LC50 >688 ppm) at the tested concentrations. The toxicity of the other tested dispersants varied between the bivalve species. For cockles, Berol TL 188, Berol TL 198, Corexit and Polyclens TS 7 produced toxicity values of 200, 450, 1,000, and >178 ppm for the 96-hour exposures, respectively. However, the mortality of the bivalves tended to occur after the end of the exposure period during the 48 hours in clean water. Therefore, when the 48-hour period was included into the LC50 values the concentrations required to cause 50% mortality were decreased. When including the 48-hour period in clean water the LC50 values for Berol TL 188 and Berol TL 198 are reduced to 130 and 270 ppm, respectively. The oil emulsion with heavy fuel oil was the most toxic to cockles with a 96-hour LC50 value of <25 ppm (the lowest concentration tested). Corexit 7664 with diesel oil, Corexit 8664 with diesel oil, and Corexit 7664 with crude oil produced 96-hour LC50 values of 140, 50, and 60 ppm, respectively. However, when the 48-hour period in clean water was included the LC50 values were 50, 40 and <50 ppm, respectively. Oman crude oil did not cause 50% mortality at the tested concentrations. However, 30% mortality occurred for cockles at the highest concentration tested concentration (1,000 ppm).

3.2 Dispersants

The effects of dispersants themselves were examined by six articles (of which three were not accessible), producing 45 results (ranked 'worst-case' mortalities). The evidence is summarized below, except for Swedmark *et al.* (1973) which is summarized above.

Nagell *et al.* (1974) investigated the toxicity of four oil dispersants to organisms from the Baltic Sea. The dispersants tested were Corexit 7664, Berol TL-188, Berol TL-198, and BP IIOO-X. The 96-hour LC50 value for *Cerastoderma glaucum* exposed to BP 1100-X was 2,000 mg/l. The toxicity values for the other oil dispersants were not reported for *Cerastoderma glaucum*.

Portmann & Connor (1968) investigated the toxicity of twelve oil-spill removers on marine shellfish. *Cerastoderma edule* were exposed to various concentrations of the dispersants and detergents for 48 hours in static conditions to establish 48-hour LC50s (the concentration of toxin required to kill 50% of the test animals) (see Table 3.1).

Table 3.1. The effects of 48-hour dispersant exposure on the survival of *Cerastoderma edule* adults (Portmann & Connor, 1968).

Dispersant	48-hour LC50 (mg/l)
Polyclens	70
Slickgone 1	32.4
Slickgone 2	30.5
Slix	12.7
Atlas 1901	48.5
BP 1002	81
BP 1002	75*
Cleanosol	19.2
Dermol	148
Essolvene	63
Gamlen CW	69.5
Gamlen D	38.8
Gamlen OSR	15.8

*24-hour LC50

Further studies by Portmann & Wilson (1971) and Portmann (1972) were not accessible but the data was provided by ECOTOX[4] and included in the 'Mollusc Evidence Summary' spreadsheet.

3.3 Petroleum hydrocarbons – phenols

The effects of phenols were examined in four articles, producing six results (ranked 'worst-case' mortalities). The evidence is summarized below.

Lusher *et al.* (2017) investigated the effects of endocrine disrupting chemicals, bisphenol-A, and 17b-oestradiol, on *Cerastoderma edule*. Cockles were exposed to nominal concentrations of 17b-oestradiol (0.1 µg/l) and bisphenol-A (0.1 and 0.01 µg/l) for 60 days. At the end of the 60 days experiment, there were no statistical differences in the condition index of the cockles. However, there were lower survival rates of cockles exposed to both bisphenol-A (BPA) concentrations in comparison to 17b-oestradiol (E2) and the control, with mortality rates of 34, 31, 18 and 19%, respectively. There were no occurrences of intersex identified from exposure to 17b-oestradiol (E2) or bisphenol-A at the tested concentrations.

Marin *et al.* (2008) investigated the lethal and sublethal effects of the xenoestrogen 4-nonylphenol on the cockle *Cerastoderma glaucum*. Cockles were exposed to a range of concentrations (190, 380, 750, 1,500, and 3000 µg/l) of 4-nonylphenol for 96 hours to establish the concentration that was lethal to 50% of the test individuals. The 96-hour LC50 value was 300 µg/l. No mortality was observed at 100 µg/l.

Portmann & Wilson (1971) reported *Cerastoderma edule* adults exposed to cresol and phenol for 48 hours produced LC50 values of >100 and >500 mg/l, respectively. Portmann (1972) reported *Cerastoderma edule* larvae exposed to cresol and phenol for 48 hours produced LC50 values of >100 and >330 mg/l, respectively.

3.4 Polyaromatic hydrocarbons (PAHs)

The effects of polyaromatic hydrocarbons (PAHs) were examined by one article.

Wootton *et al.* (2003) investigated differences in PAH-induced immunomodulation in three bivalve molluscs. The commonly used sentinel bivalve, *Mytilus edulis*, was compared with *Cerastoderma edule* and *Ensis siliqua*. The bivalves were exposed to a range of

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

phenanthrene concentrations (50, 100, 200, or 400 µg/l) for 14 days and haemocyte immunity parameters, including haemocyte counts, phagocytosis, superoxide generation, lysosomal enzymes, and lectin-binding were monitored. The results showed that phenanthrene exposure caused different immune responses in the three bivalves. Exposure to the highest tested concentration of phenanthrene (400 µg/l) caused 100% mortality of both *Cerastoderma edule* and *Ensis siliqua* within 14 and 7 days of exposure, respectively.

3.5 Sensitivity assessment – Hydrocarbons and PAHs

The count of ranked mortalities due to 'Hydrocarbons and PAHs' are summarized in Figure 3.1 and Table 3.2 below. The data presented in Table 3.2 include all life stages and articles where life stage was not reported or were unspecified (NR).

3.5.1 Recovery rates and resilience assessment.

The recovery rates (resilience assessments) used in the sensitivity assessments below were taken from the MarLIN website, where possible.

***Cerastoderma* spp.** Patches of cockles are naturally more variable over space and time (Smaal *et al.*, 2005) and beds are subject to either gradual declines as the population aged but inhibits recruitment or occasional mass mortalities that have been attributed to a number of causes (Burdon *et al.*, 2014). In habitats where this biotope occurs, there may be dense beds of cockles with adjacent patches of sediment where the cockles have been removed (by natural decline and disturbance or fisheries). Small, disturbed, patches may be rapidly infilled by movement of adult cockles by tidal currents and wave action or active migration of adults. Therefore, when resistance is assessed as 'Medium' (25% of population or habitat removed or severely affected), **resilience is assessed as 'High'** based on migration and recovery from adjacent sediments (where the habitat remains suitable). As recruitment in *Cerastoderma edule* is episodic**, resilience is assessed as 'Medium' (2-10 years) when resistance is 'Low' (loss of 25-75% of populations and/or habitat) or None (>75% of population removed, or habitat impacted)**. It should be noted that small patches of disturbance within dense beds of cockles may recover rapidly through migration and displacement of cockles (Tillin & Tyler-Walters, 2016). The resilience assessment for *Cerastoderma glaucum* is assumed to be the same as *C edule* in the absence of other evidence.

***Macoma* spp.** *Macoma balthica* has a life span of 5-10 years, a generation time of 1-2 years and reaches maturity at 1-2 years. Hence recovery is probably rapid and complete in

approximately 2 years ('High' resilience) where resistance is High, Medium, or Low but full population recovery, **following large scale removal of a population (resistance is None) may take >2 years (resilience is 'Medium')** (Ashley, 2016.).

Table 3.2. Summary of count of worst-case ranked mortalities to 'Hydrocarbons and PAH' contaminants reported in the evidence review and resultant proposed sensitivity assessments for mollusc 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
Cerastoderma edule											
	Mixtures										
		Dispersant	**9**	34				43	N	M	M
		Oil emulsion	**4**					4	N	M	M
	Petrochemical										
		Phenols		**6**				6	L	M	M
		Complex hydrocarbons	**1**					1	N	M	M
	Pyrogenic										
		PAHs	**1**					1	N	M	M
Total			**15**	**40**				**55**	**N**	**M**	**M**
Cerastoderma glaucum											
	Mixtures										
		Dispersant		**1**				1	L	M	M
	Petrochemical										
		Phenols		**1**				1	L	M	M
Total				**2**				**2**	**L**	**M**	**M**
Macoma balthica											
	Mixtures										
		Dispersant					1	1	H	H	NS
Total							**1**	**1**	**H**	**H**	**NS**
Overall total			**15**	**41**			**1**	**57**			

***Scrobicularia* spp.** The population dynamics of *Scrobicularia plana* is marked by variation in recruitment depending on temperature, latitudinal gradient, and local habitat condition, together with high post-recruitment mortality, especially in winter months (Essink *et al.*, 1991; Verdelhos *et al.*, 2011). In southern areas, two or three cohorts of recruits occurred annually whereas only one was found in northern sites (Essink *et al.*, 1991; Verdelhos *et al.*, 2011). Good recruitment resulted in high abundances, but their abundance did not last more than two to three years, with strong reductions in numbers after winter (Essink *et al.*, 1991). Recruitment failures were also noted during the first four years after a severe winter (Essink *et al.*, 1991). Essink *et al.* (1991) suggested that subtidal populations, although limited, may provide a supply of recruits as they were less affected by winter temperatures. Essink *et al.* (1991) also noted that the longevity of adults (up to 16 years) might mean that the population could survive recruitment failure if enough adults survived to produce many larvae each year. (Verdelhos *et al.* (2011) suggested the existence of different life strategies within populations of *Scrobicularia plana*. Higher-latitude populations usually exhibited low abundance values, shorter reproduction periods, and lower growth rates. Therefore, as recruitment in *Scrobicularia plana* is variable**, resilience is assessed as 'Medium' (2-10 years) when resistance is 'Low' (loss of 25-75% of populations and/or habitat) or None (>75% of population removed, or habitat impacted)** but where resistance is assessed as 'Medium' (25% of population or habitat removed or severely affected), **resilience is assessed as 'High'.**

3.5.2 *Cerastoderma* spp.

Studies of the effects of Hydrocarbons or PAHs', on *Cerastoderma edule* contributed 98% of the results (worst-case ranked mortalities) on this pressure, of which 27% reported 'Severe' mortality and 73% reported 'Significant' mortality. Most (74%) of the results were from studies that examined the effects of dispersants and emulsified oils, which reported 'Severe' or 'Significant' mortality in all cases. 'Severe or 'Significant' mortality was also reported due to exposure to complex hydrocarbons, phenols, and a PAH (phenanthrene) in *C. edule*. 'Significant mortality was also reported in *C. glaucum* (Table 3.2). **Therefore, the worst-case resistance of *Cerastoderma* spp. to 'Hydrocarbons and PAHs' is assessed as 'None'. Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'.** Confidence in the assessments is assessed as 'Medium' because only the data rather than the original data sources were accessible.

3.5.3 *Macoma* spp.

Stekoll *et al*. (1980) exposed the *Macoma balthica*, to Prudhoe Bay crude oil in flowing seawater for six months at three concentrations; low 0.03 mg/l, medium 0.3 mg/l and high 3.0 mg/l and concluded that chronic exposure of *Macoma balthica* to oil-in-seawater concentrations even as low as 0.03 mg/l would in time lead to population decreases. The individuals in this study were not subjected to any of the stresses that normally occur in their natural environment on mudflats such as changes in salinity, temperature, oxygen availability and wave action, therefore, it is possible that exposure of *Macoma balthica* to oil under field conditions results in higher mortality.

Shaw *et al*. (1976) also reported mortality of *Macoma balthica* caused by exposure to crude oil following an experimental application of oil at a concentration of 1.2 µl oil/cm² and 5.0 µl oil/cm² to sediments which equated to oil spills of one ton /20 km² and one ton/100 km². 'Significant' mortalities were observed after only two days following the application of the oil at a concentration of 5.0 µl oil/cm². Some specimens of *Macoma balthica* survived the application of oil in these experiments but were weakened (Shaw *et al*., 1976).

Therefore, **the worst-case resistance of *Macoma* spp. to petroleum hydrocarbons is assessed as 'Low', resilience as 'High' and sensitivity as 'Low'** but with 'Low' confidence due to the limited number of studies reviewed.

Only one article (Farke & Gunther, 1984) examined the effects of dispersants on *Macoma balthica*. No mortality was reported, and it was unclear what effects the dispersant had on the population based on the data alone. **Therefore, the evidence was not adequate to support an assessment of its sensitivity to dispersants.**

3.5.4 *Scrobicularia* spp.

No results on the impacts of 'Hydrocarbons and PAHs' on *Scrobicularia plana* were found in the evidence reviewed, and no sensitivity assessment was made.

4 Transitional metals and organometals

A total of 100 results (ranked 'worst-case' mortalities) were obtained from 53 articles that examined the effects of transitional metals and organometals on the selected mollusc species reviewed (Figure 4.1 and Figure 4.2). *Macoma* (syn. *Limecola*) was the most studied with 42% of the results, followed by *Scrobicularia plana* with 31% and then *Cerastoderma* spp., with 27%.

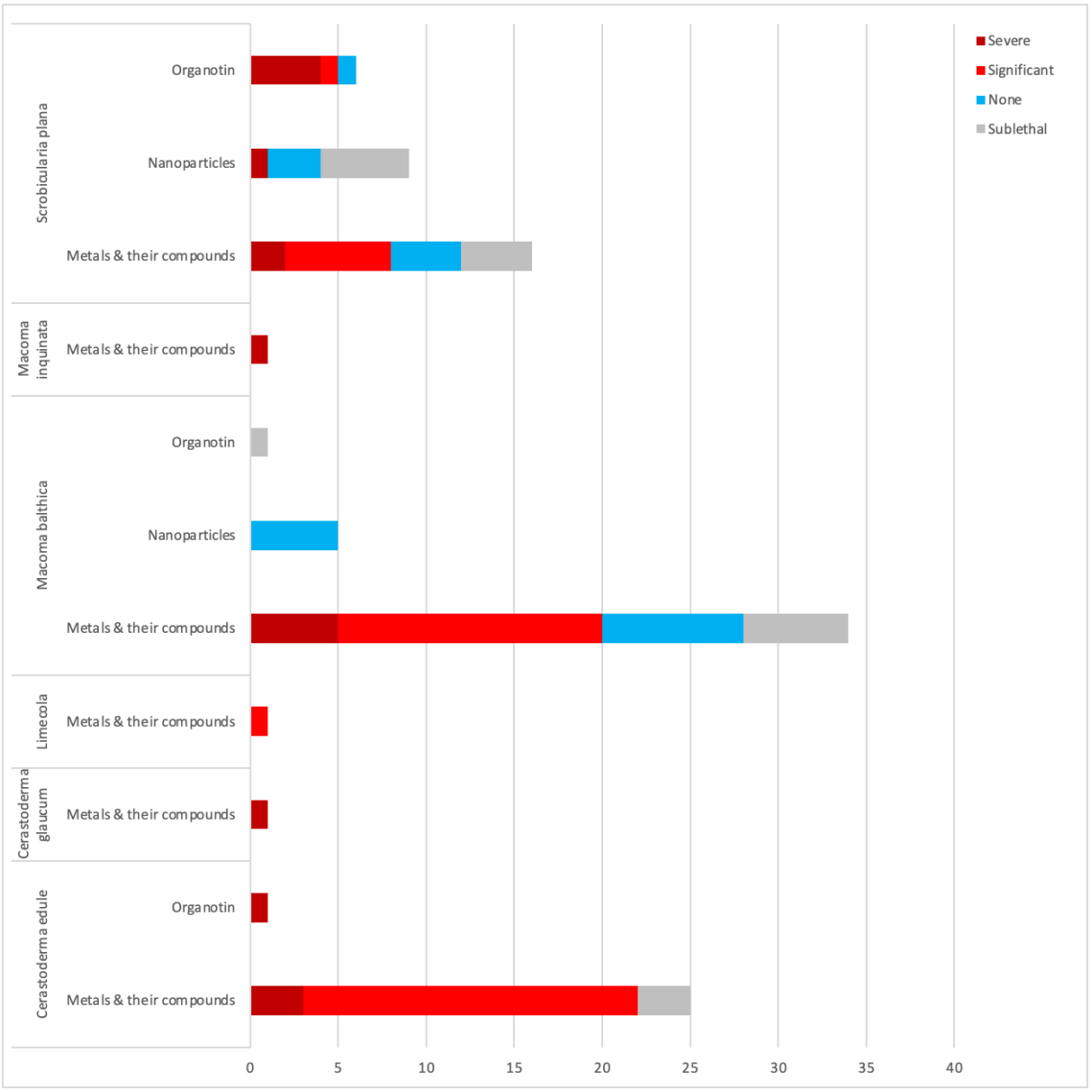

Figure 4.1. Count of worst-case ranked mortalities due to exposure to metals, nanoparticulates, and organometals in selected mollusc species. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects.

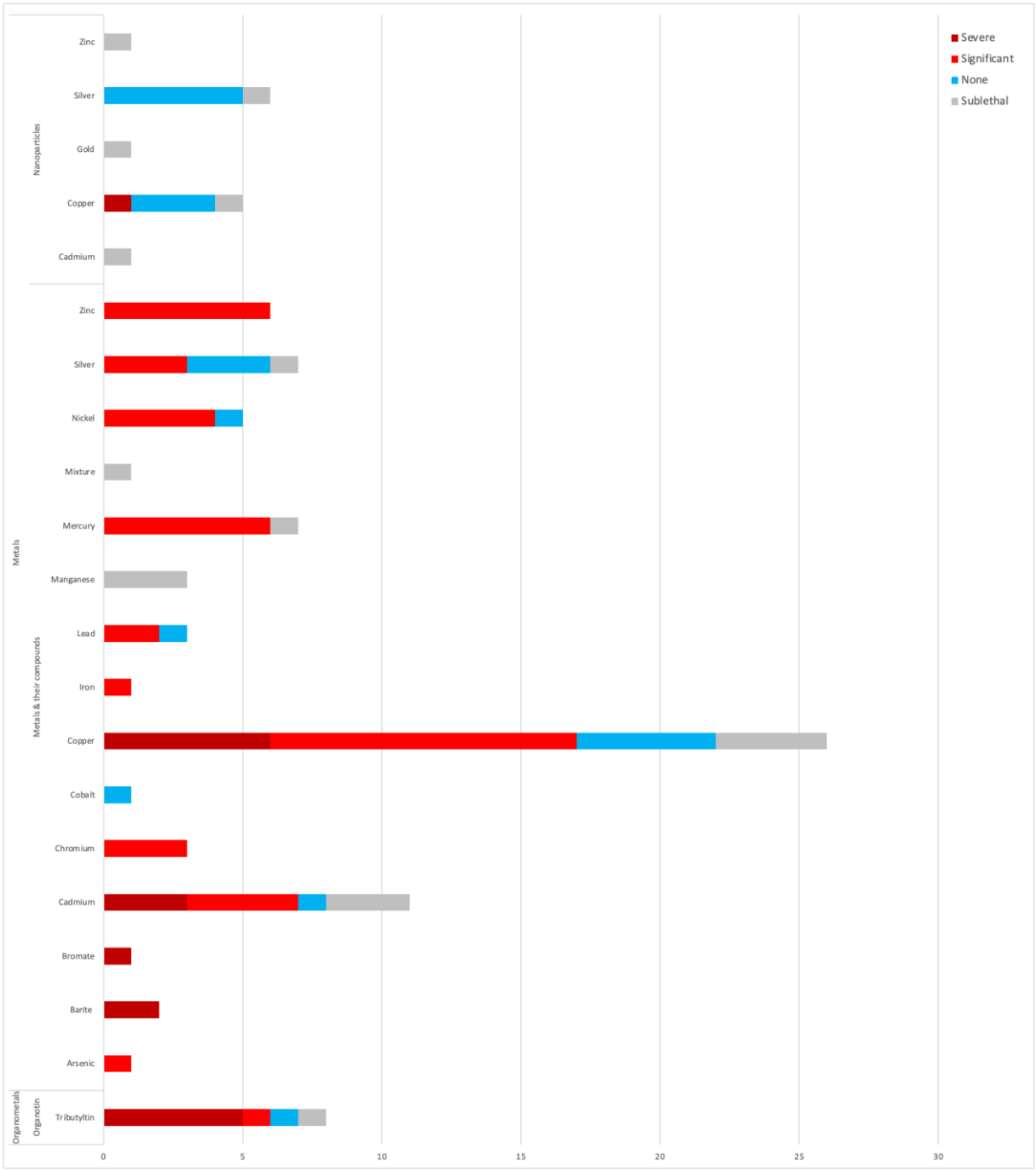

Figure 4.2. Count of worst-case ranked mortalities due to exposure to metals, nanoparticulates, and organometals in selected molluscs. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

4.1 Transitional metals

The most studied metals were in the order copper (31%) >silver (13%) >cadmium (12%) (Figure 4.2). The effects of exposure to transitional metals resulted in 59% mortality, 22% in ‘no’ mortality and 19% in sublethal effects. The evidence for the articles that reported

mortality is summarized below, apart from the evidence from Dai *et al.*, 2013, Buffet *et al.*, 2013b, and Buffet *et al.*, 2014, which is reported in nanoparticulate metals.

Absil *et al.* (1996) exposed *Macoma balthica* individuals to dissolved copper at a concentration of 25 µg/l daily for 18 days in a flow-through system in fed and unfed experiments. The 18 days of exposure was followed by a 35-day period without any copper addition. Mortality occurred after seven days in both the fed and unfed experiments, and mortality continued to occur in the 35 days without copper with mortalities of 32% for the fed individuals and 45% mortality for the unfed individuals after 40 days. No mortalities occurred in the control treatment.

Akberali & Black (1980) exposed *Scrobicularia plana* to 10, 50, 100 and 500 µg/l copper until 50% mortality had occurred. Mortality occurred in the highest copper treatment after 48 hours and the LT50 was established at 168 hours in the 500 µg/l treatment.

Amiard *et al.* (1985) reported *Scrobicularia plana* exposed to cadmium, copper, lead, and zinc for 96 hours had LC50 values of 1,600, 3,200, 43,400, and 10,000 µg/l, respectively. Data retrieved from ECOTOX. Original source text not accessible.

Ballan-Dufrancais *et al.* (2001) exposed the Baltic clam *Macoma balthica* from clean and metal contaminated sites to 30 ng/l or 40 ng/l for 13 days. No mortality occurred in any of the treatments.

Barlow & Kingston (2001) exposed *Cerastoderma edule* and *Macoma balthica* to daily doses of 1, 2, and 3-mm depth equivalents of barite for 12 days. Total (100%) mortality occurred within 12 days for both species at the 2- and 3-mm depth dosage. They determined a 4-day LD50 of 2.6 mm in *C. edule* that fell to 1.5 mm after eight days. *M. balthica* was more tolerant with a 4-day LD50 of 2.8 mm that fell to 2 mm after eight days. In both species, mortality was caused by damage to the gill filaments.

Berthet *et al.* (1992) exposed *Scrobicularia plana* to concentrations of silver between 1 to 1000 µg/l for 16 days. *Scrobicularia plana* had 50% mortality that occurred within 3 days at the highest tested concentration (1,000 µg/l), 4 days at 200 µg/l and 10.4 days at 100 µg/l. At the lowest concentrations of silver (<50 µg/l) 50% mortality was not reached within the 16 days experiment, therefore the LT50 value is >16 days.

Bertrand *et al.* (2016) exposed *Scrobicularia plana* to silver nitrate (10 µg/l) or silver nanoparticles (10 or 20 µg/l) at 15 and 30% salinity for seven days. No mortalities and no

differences in condition index were recorded for any of the exposures, during the seven-day period.

Boisson *et al.* (1998) exposed *Macoma balthica* from a contaminated and uncontaminated site to 80 µg/l silver or 100 µg/l mercury for 15 days to establish the time for which 50% of the population of clams exposed to a contaminant died (LT50). The clams from the non-polluted estuary had LT50 values of >15 days for silver and 12 days for mercury. The clams from the contaminated site had LT50 values of 9.3 days for silver and 11 days for mercury. The LT50 value for the clams from the contaminated site was significantly lower than the LT50 of the clam from the uncontaminated site.

Bryant *et al.* (1984) exposed *Macoma balthica* to chromium at different temperatures (5, 10, 15'C) and a range of salinities (5 to 40%), at time intervals of up to 384 hours. The toxicity of copper increased as temperature increased and as salinity decreased. *Macoma balthica* 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 29 to 640 mg/l chromium depending on exposure temperature and salinity.

Bryant *et al.* (1985) exposed *Macoma balthica* to arsenic at different temperatures (5, 10, 15℃) and a range of salinities (5 to 35%), at time intervals of up to 384 hours. *Macoma balthica* were exposed to arsenic concentrations of 2, 4, 8, 16, 32, 64, and 128 ppm (+ controls) and salinities of 15, 25, and 35%, for up to 192 hours. The 48-hour, 96-hour, and 192-hour LC50 values ranged from 500 to >1,000 ppm, 85 to >1,000 ppm, and 15 to 220 ppm arsenic, respectively, depending on exposure temperature and salinity. The survival of the species decreased as time, temperature and concentration of arsenic increased. However, salinity did not have any significant effects.

Bryant *et al.* (1985b) exposed *Macoma balthica* to nickel and zinc at different temperatures (5, 10, 15°C) and a range of salinities (5 to 35%), at time intervals of up to 384 hours. *Macoma balthica* were exposed to nickel at concentrations of 16, 32, 64, 128, 256, 512, 1,000, and 2,000 ppm (+ controls) and at salinities of 15, 25, and 35%, for up to 384 hours. The 24-hour, 48-hour, 96-hour, and 192-hour LC50 values ranged from 450 to >2,000 ppm, 260 to >2,000 ppm, 95 to 1,100 ppm and 65 to 450 ppm nickel, respectively, depending on exposure temperature and salinity. *Macoma balthica* were exposed to zinc at concentrations of 15, 30, 60, 125, 250, 500, 1,000 and 2,000 ppm (+ controls) and at salinities of 15, 25, and 35%, for up to 192 hours. The 24-hour, 48-hour, 96-hour, and 192-hour LC50 values ranged

from 85 to >2,000 ppm, 320 to >2,000 ppm, 60 to 950 ppm and 65 to 360 ppm zinc, respectively, depending on exposure temperature and salinity. The median survival times of the clams exposed to nickel and zinc decreased as salinity decreased. In addition, increases in temperature caused decreases in median survival when the clams were exposed to zinc but not to nickel.

Buu & Le Gal, (1989) reported *Cerastoderma edule* exposed to cadmium had a 96-hour LD50 value of 10,000 µg/l.

Crecelius (1979) reported *Macoma inquinate* exposed to bromate had a 72-hour LC100 value of 880 mg/l.

Eldon *et al.* (1980) exposed *Macoma balthica* to mercury, cadmium, copper, zinc, lead, nickel, and cobalt for 24 hours at concentrations ranging between 0.01 to 100 ppm. The clams were moved to clean aquariums for 15 days after exposure. Exposure to 1 ppm mercury caused 70% mortality within 15 days. At 0.5 ppm mercury, 15% mortality occurred. Cadmium exposure at 0.5 ppm caused no mortalities, but at 1 and 2 ppm cadmium there was 85% and 45% mortality within 15 days, respectively. Copper exposure between 0.1 to 2 ppm copper caused no mortalities. Zinc exposure at 20 and 50 ppm caused, 25% mortality and 65% after 15 days, respectively. Lead and nickel exposures up to 20 ppm caused no mortalities within 15 days. Cobalt exposures up to 100 ppm caused no mortalities within 15 days.

Foekeme *et al.* (2015) exposed *Cerastoderma edule* to copper from 1 to 31 µg/l for 82 days in an mesocosm experiments. At the end of the 82-day experiment 50% of the cockles in the control and treatments up to 9.9 µg/l copper survived. However, significant mortality of 90% and 100% occurred in the 16 and 31 µg/l treatments, respectively.

Hummel *et al.* (2001) exposed different populations of Baltic clams *Macoma balthica* to copper at 50 and 100 µg/l copper to establish the LT50s for each of the populations. Mortality occurred at both tested concentrations, with LT50 values from 12 days for 100 µg/l copper, and from 18 days for 50 µg/l copper. The rate of mortalities did not significantly differ from the different locations.

Kaitala (1988) exposed *Macoma balthica* to copper for 10 days. After the exposure, the clams were transferred to an aquarium with clean sediment, the clams that did not burrow were considered dead. The 10-day EC50 was established at 54 mg/l.

Ladhar-Chaabouni *et al.* (2009) exposed the cockle *Cerastoderma* glaucum to cadmium at various concentrations (50, 75, 100, 150 µg/l) for several periods of time (5, 10, 15 and 20 days) before being exposed to anoxia by air exposure. The LT50 from exposure to cadmium and anoxia ranged between 2 to 6.58 days depending on concentration and exposure time. The higher the exposure concentrations and exposure times the lower the LT50s.

Lopes *et al.* (2014) investigated the ecological effects of metal contaminated sediments, dominated by mercury and arsenic, following a decade of no industrial effluent emissions. *Scrobicularia plana* were exposed to contaminated sediment from various locations of an impacted site for 28 days. Results showed the highest bioaccumulation and mortality in the most contaminated sediments with mortalities up to 75%. There were no significant differences in the weight or length of *Scrobicularia plana* after 28 days of exposure.

Luoma *et al.* (1983) exposed *Macoma balthica* from different populations to copper at concentrations between 10 to 1,900 µg/l in static ten-day toxicity experiments, to establish LC50 values. No mortality occurred during the ten days of exposure at the lowest tested concentration (10 µg/l). However, mortality rates exceeded 50% from 500 µg/l in some of the tested populations.

McLeese & Ray (1986) exposed *Macoma balthica* to cadmium at concentrations between 0.05 and 50 mg/l and to copper at concentrations between 0.05 and 30 mg/l for 96 hours. The 144-hour LC50s for *Macoma balthica* exposed to cadmium and copper were 2.8 and 6 mg/l, respectively.

Mesquita *et al.* (2018) exposed the bivalves *Cerastoderma edule* and *Scrobicularia plana* of different sizes (small and large) to copper sulphate for 96 hours. *Cerastoderma edule* were exposed to 600, 900, 1,200, 1,500, 1,800 and 2,100 µg/l copper sulphate and *Scrobicularia plana* were exposed to 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, and 4,000 µg/l copper sulphate. The results of the lethality tests showed that *Cerastoderma edule* was more sensitive to copper exposure than *Scrobicularia plana* with LC50 (L) = 818 µg/l, LC50(S)=1,129 µg/l and LC50(L)= 2,563 µg/l, LC50(S)= 4,705 µg/l, respectively. In addition, the results show that larger individuals are more sensitive than smaller ones.

Mouneyrac *et al.* (2000) exposed *Macoma balthica* from a polluted site and clams from a control site to various metals. The clams were exposed to cadmium (0.01 µg/l), silver (0.01, 0.05 and 0.1 µg/l) and mercury (100 µg/l) until 50% mortality had been observed. Mortality was recorded daily, and the mean lethal times (LT50) were calculated. No mortality was

observed after 10 days in the cadmium or silver treatment at 0.01 µg/l. In the 0.05 and 0.1 µg/l silver treatments, the LT50 was 8.6 and 5.6 days, respectively, in clams from the polluted site and 10.4 and 4.5 days in clams from the control site. In the 0.1 µg/l mercury treatment the LT50 s were to 8.0-12.2 and 8.7 days for the polluted and clean site, respectively.

Naylor (1987) reported *Cerastoderma edule* exposed to copper for six days had a NR-LETH value of 100 µg/l. *Cerastoderma edule* exposed to zinc for ten days had a LC50 value of 10,000 µg/l.

Neuhoff & Theede (1984) exposed the bivalves *Macoma* calcarean and *Macoma balthica* to copper at concentrations between 10 and 300 ug/dm-3 in a renewal experiment. The time of exposure leading to 50% mortality (LT50) was recorded. At 30 ug/dm-3 the LT50 values for *Macoma* calcarean and *Macoma balthica* were 37 and 62 days, respectively. The polychaetes were more sensitive with LT50s of 22 and 37 days.

Portmann (1968) exposed *Cerastoderma edule* to copper, zinc, mercury, phenol, and nickel for 48 hours to establish the concentrations that caused 50% mortality. The most toxic was mercury, followed by copper (1 mg/l), zinc (<300), phenol (>500) and nickel (>500). Additionally, further testing showed exposure to copper and mercury, at reduced temperatures from 22 to 5 C increased the toxicity of the tested substances. In addition, the differences using fed and unfed cockles was investigated using mercury toxicity, the LD50 for cockles was reduced when using unfed cockles with LD50 of 15.5 mg/l for fed and 9.6 mg/l for unfed. Further studies by Portmann & Wilson (1971) and Portmann (1972) were not accessible but the data was provided by ECOTOX[5] and included in the 'Mollusc Evidence Summary' spreadsheet.

Ruiz *et al.* (1994) exposed *Scrobicularia plana* spat to TBT and copper over 30-day periods. The TBT test concentrations were 0.5, 1, 2, and 4 µg/l. The results showed that the concentration required to kill 50% of the individuals was <1.3 µg/l. In the copper tests, the concentrations were 10, 20, 40, and 80 µg/l. Mortalities did not increase significantly compared to the control.

Ruiz *et al.* (1996) exposed *Scrobicularia plana* larvae to copper at 5, 10, 20, and 40 µg/l for 48 hours in static conditions. The percentage of D-larvae in the 5 and 10 µg/l treatments were similar to the control. However, in the 20 µg/l treatment the number of D-larvae was

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

significantly reduced by 90% but no D-larvae were produced in the 40 µg/l treatment. In the 20 µg/l treatment, 65% of those D-larvae that developed were abnormal and there were reductions in shell lengths. There was no difference in the percentage of abnormalities and length between the 5 and 10 µg/l copper when compared to the control.

Scola *et al.* (2021) exposed *Scrobicularia plana* to sediment spiked with copper oxide nanoparticles (CuO NPs) and soluble copper (CuCl2). The clams were exposed to sediment spiked with 50 and 500 ug/g of copper for 30 days. Results showed both high copper exposure treatments (500 ug/g) resulted in 100% mortality within 30 days of exposure. Mortality was not linked with the different forms of copper exposure.

Sharov *et al.* (2022) exposed *Limecola balthica* to 0, 100, 500, and 5000 µg/l cadmium for ten days in static exposures. ‘Significant’ mortality (>50%) occurred at 5,000 µg/l cadmium.

Sokolowski *et al.* (1999) exposed *Macoma balthica* from two sites (polluted and unpolluted) to cadmium (37.5 and 75 µg/l) for up to two months. The clams from the relatively unpolluted site were more sensitive to copper exposure with the highest mortalities. The LT50 values of clams from the polluted site at 35 µg/l was 59 days, and the LT50 of clams from the relatively unpolluted site was 51 days. In the 75 µg/l treatment, the LT50 of clams from the polluted site was 41 days, and the LT50 of clams from the relatively unpolluted site was 33 days.

4.2 Organometals

Tributyltin (TBT) was the only studied organometal with eight results from six articles. The evidence is summarized below.

Beaumont *et al.* (1989) conducted a four-month microcosm experiment to investigate the toxicity of tributyl tin (TBT) on marine organisms. The setup contained a series of sandy-substrate 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) and three at low (0.06-0.17 µg/l) concentrations. At high levels of TBT, all *Cerastoderma edule* died within two weeks and progressive mortality was observed in the low TBT treatments over time with 80% mortality after 17 weeks. *Scrobicularia plana* mortalities increased with time in the high TBT treatments, with 100% mortality after 10 weeks of exposure. 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 *Macoma balthica* juveniles occurred in the low-level TBT and the control treatments, with no significant differences found between number or size.

Ruiz *et al.* (1994) investigated the effects of chronic toxicity of TBT and copper on the spat of *Scrobicularia plana*. Toxicity tests were conducted over 30 days, the first test carried out in 1991 investigated the effects of tributyltin (TBT) and copper on survival and burying activity. The TBT the test concentrations were 0.5, 1, 2, and 4 µg/l. The results showed that the concentration required to kill 50% of the individuals was <1.3 µg/l and burrowing activity was impaired significantly from day six in all TBT treatments. The copper, the test concentrations were 10, 20, 40 and 80 µg/l. Mortality did not increase significantly compared to the control. However, increased burrowing time was observed at concentrations at or above 20 µg/l. The second test in 1992 investigated TBT effects on the growth and burying activity at concentrations of 50, 125, 250 and 500 ng/l. The growth rate was significantly reduced by all TBT concentrations, but burrowing rates were inconsistent.

Ruiz *et al.* (1994b) investigated the toxicity of tributyltin polluted sediment on the spat of the bivalve *Scrobicularia plana*. Toxicity bioassays were run for 36 days, and lethal and sublethal effects were investigated. The TBT-polluted sediment did not produce any mortality or avoidance behaviour response, but the growth and burying activity of clams were significantly reduced at the end of the experimental period when compared to the control treatments.

Ruiz *et al.* (1995) investigated the toxicity of tributyltin on pediveliger larvae of the bivalve *Scrobicularia plana*. A static renewal test was run for 30 days at 50, 125, 250, and 500 ng/l tributyltin to assess the impacts on the survival and growth of the larvae. Exposure to TBT levels of >125 ng/l resulted in significant mortalities (>50 %) and negligible shell growth of individuals. Larval shell growth was reduced significantly and abnormal at the lowest concentration tested (50 ng/l).

Ruiz *et al.* (1995b) investigated the toxicity of tributyltin on the embryonic development of the bivalve *Scrobicularia plana*. Embryos were exposed to TBT at 50, 125, 250, 500, and 1,000 ng/l for 48 hours for four hours after fertilization. The hatch rate of the larvae was influenced significantly by 250 ng/l, and 100% hatch rate inhibition/embryo death occurred at 1,000 ng/l. Exposure to TBT caused abnormal larvae at 9%, 51% and 85% in the 125, 250, and 500 ng/l treatments, respectively. The mean length of the D-larvae produced in the 250 and 500 ng/l

treatments were reduced compared to the D-larvae in the controls and at the lowest TBT treatment.

Ruiz *et al.* (1995c) investigated the toxicity of tributyltin (TBT) on the development of veliger larvae of the bivalve *Scrobicularia plana*. Larvae were exposed to TBT at 50, 125, 250, and 500 ng/l for ten days. The growth of the exposed larvae was reduced significantly compared to the controls. Survival in all of the exposure treatments were reduced significantly compared to the seawater control (70% survival), with survival rates between 0 to 30% in the exposure treatments.

4.3 Nanoparticulate metals

Ten articles examined nanoparticulate (NP) metals. The evidence is summarized below.

Bertrand *et al.* (2016) investigated the influence of salinity and silver (standard and nanoparticulate) on the bivalve *Scrobicularia plana*. The bivalves were exposed to silver nitrate (10 µg/l) or silver nanoparticles (10 or 20 µg/l) at 15 and 30% salinity for seven days. No mortalities and no differences in condition index were recorded for any of the exposures, during the seven-day period.

Buffet *et al.* (2011) investigated the effects of copper oxide nanoparticles on the behavioural and biochemical responses of *Scrobicularia plana* and *Hediste diversicolor*. The clams 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 clams were assessed using 20 individuals from each treatment. After four days of exposure, the burrowing tests were conducted by placing the clams onto sediment and recording the number that had burrowed at frequent intervals: every 5 min in the 1st hour, every 10 min in the 2nd hour, every 20 min in the 3rd and 4th hour, then every hour until six or seven hours of the test. The feeding tests were conducted after eleven days of exposure. The clams were fed *Tetraselmis suecica* for an hour after which time the concentration of algae not ingested was measured. The results from the burrowing test showed that the clams exposed to soluble or nanoparticulate copper had reduced burrowing times compared to the controls. The feeding rate of the clams was significantly reduced by exposure to copper oxide nanoparticulates.

Buffet *et al.* (2012) investigated the effects of zinc oxide nanoparticles in sediment on the feeding rate and burrowing behaviour of the clam *Scrobicularia plana*. 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. Burrowing tests were conducted after seven days of exposure to one of the three treatments. Individuals were placed onto sediment and the number that had burrowed at certain time intervals was recorded (every 5 min in the first hour, every 10 min in the second hour, every 20 min in the third and fourth hour, and then every hour until six or seven hours of the test). The feeding rate of the clams was estimated following 10 days of exposure to the treatments. The feeding rate was established by calculating the concentration of *Tetraselmis suecica* left after an hour of feeding. Exposure to zinc oxide nanoparticles reduced both the burrowing time and feeding rates of the clams.

Buffet *et al.* (2013) investigated the biochemical and behavioural responses of *Scrobicularia plana* to silver (soluble and nanoparticulate) in seawater and microalgal food. The clams were exposed to three treatments; natural seawater only; soluble silver at 10 µg/l or silver NPs at 10 ug/ for 14 days, either via food or via water column. Behavioural tests were conducted via burrowing and feeding rate tests. Burrowing tests were conducted on day 6 for direct exposure and day 5 for food exposure. Feeding rate tests were performed after 13 days for waterborne exposure and after 10 days of dietary exposure. The burrowing of the clams was not affected by any of the silver exposure treatments. However, the feeding rate was impaired after 10 days of dietary exposure. Waterborne exposure of bivalves to either form of silver did not affect the feeding rate.

Buffet *et al.* (2013b) investigated the effects of copper oxide nanoparticles and copper nitrate on the feeding rate and burrowing behaviour of the ragworm *Hediste diversicolor* and the clam *Scrobicularia plana*, in environmentally realistic conditions in outdoor mesocosms. The burrowing and feeding rate of the clams were tested after 21 and 14 days of exposure to 10 µg/l copper in either soluble or nanoparticulate form. The burrowing rate of the clams was significantly slower when exposed to either form of copper compared to the controls. In addition, the burrowing rate was significantly lower in the soluble copper treatments than in the nanoparticulate copper treatments. The feeding rates of the clams were affected significantly affected compared to the control and the soluble copper treatments by exposure to copper oxide nanoparticles.

Buffet *et al.* (2014) investigated the effects of silver nanoparticles and silver nitrate on the feeding rate and burrowing behaviour of the ragworm *Hediste diversicolor* and the clam *Scrobicularia plana* in environmentally realistic conditions in outdoor mesocosms. The

burrowing and feeding rate were tested after 21 and 14 days of exposure to silver at 10 µg/l. The feeding rate of the clams 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. No mortality was reported during the 21 days of exposure and the condition index of the clams did not show any significant differences.

Buffet *et al.* (2015) investigated the effects of cadmium sulphide (CdS) quantum dots on the oxidative stress and behaviour of the clam *Scrobicularia plana*. Behavioural tests were conducted after 14 days of exposure 10 µg/l CdS quantum dots and to 10 µg/l soluble cadmium. The behavioural patterns of the clams were impaired by exposure to CdS quantum dots with reductions in foot movements, however, soluble cadmium did not affect food movement. The feeding rates of the clams were significantly reduced after exposure to both forms of cadmium.

Dai *et al.* (2013) investigated the effects of silver, silver oxide nanoparticulates, copper and copper oxide nanoparticulates on *Macoma balthica*. Clams were exposed to sediment spiked with 200 ug/g of silver or copper for 35 days. No significant effects on mortality, condition index, or burrowing behaviour were observed for any of the metal forms.

Pan *et al.* (2012) exposed the marine bivalve *Scrobicularia plana* to gold nanoparticles of three different sizes (5, 15, and 40 nm) during a 16-day laboratory exposure at 100 mg/l. Biochemical and behavioural responses were assessed. After seven days of exposure the influence of gold nanoparticles on the burrowing behaviour was tested by placing the clams onto clean sediment and observing the number of individuals burrowed after regular time intervals (every 5 minutes during the 1st hour, every 10 minutes during the 2nd hour, every 20 minutes during the 3rd to 6th hours). At the end of the test, the number of unborrowed clams were counted. Feeding rate tests were conducted after 14 days of exposure, by feeding green algae *Tetraselmis suecica* and measuring the concentration in the water column after initial addition and at hourly intervals. Exposure to gold nanoparticles was influenced burrowing significantly after 7 days of exposure. The results showed the size of the gold nanoparticles influenced the burrowing with larger sizes of gold nanoparticles having stronger inhibition of burrowing. No significant differences in feeding behaviour were observed compared to controls after 14 days of exposure.

Scola *et al.* (2021) investigated the bioaccumulation, subcellular distribution, and toxic effect of copper oxide nanoparticles (CuO NPs) and soluble copper ($CuCl_2$) exposure on *Scrobicularia plana*. The clams were exposed to sediment spiked with 50 and 500 µg/g of

copper for 30 days. The condition index of the clams was monitored at regular (1, 5, 15 and 28 days) intervals throughout the exposure. Results showed a significant decline in condition index value over time within treatments including in the control. Both high copper exposure treatments (500 ug/g) resulted in 100% mortality within 30 days of exposure, irrespective of the form of copper. Mortality was not linked with the different forms of copper exposure. Scola *et al.* (2021) noted that mortality was delayed in specimens exposed to nanoparticulate copper, which suggested that the mechanism of toxicity was different between the different forms tested.

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,Figure 4.2, and Table 4.1 below. The data presented in Table 4.1 include all life stages and articles where life stage was not reported or were unspecified (NR). Mortality was reported in 51% of the results for *Macoma/Limecola*, 45% of the results for *Scrobicularia plana* 45% and 89% of the results for *Cerastoderma* spp.

The effects of 'metals' exposure varies with species, the metal and its compound, its exposure concentration and duration, and with environmental conditions such as temperature and salinity (for example Bryan, 1984; Bryant *et al.*, 1984, 1985a&b) (Table 4.1). However, Bryan (1984) stated that mercury is the most toxic metal to bivalves.

4.4.1 *Cerastoderma* spp.

'Severe' or 'Significant' mortality was reported in 88% of the results from studies of the effects of 'Transitional metals' exposure on *Cerastoderma edule* depending on the exposure concentration or duration of all the 'metals' studied, except for manganese (Mn). Bryan (1984) suggested that many polychaetes were resistant to heavy metals and evidence from the work of Bryan & Gibbs (1983) in the metal polluted Fal estuary supports this view. Bivalves, on the other hand, including *Cerastoderma edule* displayed a much lower tolerance and were found to be the most obvious absentees from the polluted Restronguet Creek area of the Fal (Bryan & Gibbs, 1983). Adult *Cerastoderma edule* were found to be more tolerant to metal toxicity than the juvenile or larval stages which appear unable to withstand the high concentrations of copper and zinc. Studies of *Cerastoderma edule* transplanted from polluted and uncontaminated sites resulted in 10-15% mortality within 63 days but 100% within 4 months at the Restronguet Creek (Bryan & Gibbs, 1983). Bryan & Gibbs (1983) stated that

Cerastoderma edule takes up heavy metals mainly from solution rather than from sediment and that it was excluded from Restronguet Creek by the high levels of Cu and Zn. Bryan (1984) stated that Hg was the most toxic metal to bivalves. In addition, Cu and Zn are believed to inhibit the settlement of juvenile *Cerastoderma edule*, leading to patchy distributions (Langston *et al*., 2003). Studies of *Cerastoderma edule* populations from polluted and un-contaminated sites in Southampton Water showed that tissue heavy metal concentrations were lower in summer than winter/spring, tissue heavy metal concentrations decreased with size of the cockle, and that cockles in sediments contaminated with metals and hydrocarbons had lower life expectancies, growth rates and body condition index (Savari *et al*., 1991a,b).

Barite (in the form of drilling mud barite) was shown to cause 100% mortality of *C. edule* within 12 days at a depth of 2- and 3-mm dosage but the cause may have been due to physical damage of their gill filaments rather than chemical toxicity (Barlow & Kingston, 2001). Ladhar-Chaabouni *et al.* (2009) was the only article to examine *C. glaucum* but reported that cadmium (Cd) exposure resulted in 100% mortality 18 days after exposure to 100 µg/l Cd.

Overall, the **evidence suggests that the worst-case resistance of *Cerastoderma* spp. to 'transitional metals' exposure is 'None'. Therefore, resilience is assessed as 'Medium' and sensitivity as 'Medium'.**

Only one article examined the effects of tributyltin (TBT) exposure in *Cerastoderma edule* (Beaumont *et al.*, 1989). In their flow-through mesocosm studies, all *Cerastoderma edule* died within two weeks at high concentrations of TBT (1-3 µg/l) and progressive mortality was observed in the low TBT treatments (0.06-0.17 µg/l) over time with 80% mortality after 17 weeks. Therefore, **the worst-case resistance of *Cerastoderma* spp. to TBT exposure is assessed as 'None', resilience as 'Medium' and sensitivity as 'Medium', especially long-term exposure.** However, confidence in the assessment is 'Low' due to the limited number of studies reviewed.

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

Species	Group/Type	Chemical name	Severe	Significant	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Cerastoderma edule										
	Metals									
		Barite	**1**				1	N	M	M
		Cadmium		**2**		1	3	L	M	M
		Chromium		**2**			2	L	M	M
		Copper	**2**	4			5	N	M	M
		Iron		**1**			1	L	M	M
		Lead		**1**			1	L	M	M
		Manganese				**1**	1	H	H	NS
		Mercury		**3**		1	4	L	M	M
		Nickel		**3**			2	L	M	M
		Zinc		**3**			2	L	M	M
	Organotin									
		Tributyltin	**1**				1	N	M	M
Total			**4**	**19**		**3**	**26**	**N**	**M**	**M**
Cerastoderma glaucum										
	Metals									
		Cadmium	**1**				1	N	M	M
Total			**1**				**1**	**N**	**M**	**M**
Macoma balthica										
	Metals									
		Arsenic		**1**			1	L	H	L
		Barite	**1**				1	N	M	M
		Cadmium	**2**	**1**	1	1	4	N	M	M
		Chromium		**1**			1	L	H	L
		Cobalt			**1**		1	H	H	NS
		Copper	**2**	4	3	3	12	N	M	M
		Lead			**1**		1	H	H	NS
		Manganese				**2**	2	H	H	NS
		Mercury		**3**			3	L	H	L
		Mixture[6]				**1**	1	H	H	NS
		Nickel		**1**	1		2	L	H	L
		Silver		**2**	1		3	L	H	L
		Zinc		**2**			2	L	H	L

[6] heavy metal contaminated sediment (McGreer, 1979)

Species	Group/Type	Chemical name	Severe	Significant	None	Sublethal	Total	Resistance	Resilience	Sensitivity
	Nanoparticles									
		Copper			**2**		2	H	H	NS
		Silver			**3**		3	H	H	NS
	Organotin									
		Tributyltin				**1**	1	H	H	NS
Total			**5**	**14**	**13**	**8**	**40**	**N**	**M**	**M**
Scrobicularia plana										
	Metals									
		Cadmium		**1**		1	2	L	M	M
		Copper	**2**	2	2	2	8	N	M	M
		Lead		**1**			1	L	M	M
		Silver		**1**	2	1	4	L	M	M
		Zinc		**1**			1	L	M	M
	Nanoparticles									
		Cadmium				**1**	1	H	H	NS
		Copper	**1**		1	1	3	N	M	M
		Gold				**1**	1	H	H	NS
		Silver			**2**	1	3	H	H	NS
		Zinc				**1**	1	H	H	NS
	Organotin									
		Tributyltin	**4**	1	1		6	N	M	M
Total			**7**	**7**	**8**	**9**	**31**	**N**	**M**	**M**
Total			**18**	**42**	**21**	**20**	**100**			

4.4.2 *Macoma* spp.

‘Severe’ or ‘Significant’ mortality was reported in 52% of the results from studies of the effects of ‘Transitional metals and organometal’ exposure on *Macoma* spp. depending on the exposure concentration or duration and environmental conditions. Copper and cadmium were reported to result in ‘Severe’ mortality, while arsenic, chromium, mercury, silver, zinc, and nickel were reported to result in ‘significant’ mortality. The remaining metals were reported to result in no mortality or sublethal effects (Table 4.1). As above, barite (in the form of drilling mud barite) was shown to cause 100% mortality of *M. balthica* within 12 days at a depth of 2- and 3-mm dosage but the cause may have been due to physical damage of their gill filaments rather than chemical toxicity (Barlow & Kingston, 2001).

Overall, the **evidence suggests that the worst-case resistance of *Macoma* spp. to 'transitional metals' exposure is 'None'. Therefore, resilience is assessed as 'Medium' and sensitivity as 'Medium'.**

As above, only one article examined the effects of tributyltin (TBT) exposure in *Macoma balthica* (Beaumont *et al.*, 1989). However, unlike *Cerastoderma edule*, no mortality was reported and some juvenile *Macoma* recruited to the low (0.06-0.17 µg/l) TBT treatment mesocosm. Therefore, **the worst-case sensitivity of *Macoma* spp. to TBT exposure is assessed as 'Not sensitive'** but confidence in the assessment is 'Low' due to the limited number of studies reviewed.

Dai *et al.* (2013) investigated the effects of silver, silver oxide nanoparticulates, copper and copper oxide nanoparticulates on *Macoma balthica*. Clams were exposed to sediment spiked with 200 µg/g of silver or copper for 35 days. No significant effects on mortality, condition index, or burrowing behaviour were observed for any of the metal forms. **Therefore, the sensitivity of *Macoma* spp. to nanoparticulate copper or silver is assessed as 'Not sensitive'** but confidence in the assessment is 'Low' due to the limited number of studies reviewed.

4.4.3 *Scrobicularia* spp.

'Severe' or 'Significant' mortality was reported in 45% of the results from studies of the effects of 'Transitional metals and organometal' exposure on *Scrobicularia plana* depending on the exposure concentration or duration. Copper was reported to result in 'Severe' mortality, while cadmium, lead, silver, and zinc were reported to result in 'Significant' mortality, depending on concentration, duration, and environmental conditions (Table 4.1).

Laboratory tests in clean water can be misleading as these do not reflect lowered toxicity in the marine environment due to the buffering effects of carbon and sulphide, which render copper non-labile (not bioavailable) and the influence of water pH, hardness, temperature and salinity etc. Field surveys have found that *Scrobicularia plana* is present in the highly contaminated Fal Estuary where levels of copper and zinc are high (Bryan & Gibbs, 1983). Nevertheless, the **evidence suggests that the worst-case resistance of *Scrobicularia* spp. to 'transitional metals' exposure is 'None'. Therefore, resilience is assessed as 'Medium' and sensitivity as 'Medium'.**

The effects of tributyltin (TBT) on *Scrobicularia plana* were investigated by six articles (Beaumont *et al.*, 1989; Ruiz *et al.*, 1994a&b, 1995a,b&c) and reviewed by Langston (2020).

Beaumont *et al.*, 1989 reported that *Scrobicularia plana* mortalities increased with time in the high TBT treatments (1-3 µg/l), with 100% mortality after 10 weeks of exposure. Ruiz *et al.* (1994) reported an LC50 of *Scrobicularia plana* spat of <1.3 µg/l TBT and that TBT reduced their growth rate significantly at 50, 125, 250 and 500 ng/l TBT. Ruiz *et al.* (1995) reported that exposure to TBT levels of >125 ng/l resulted in significant mortalities (>50%) of *S. plana* pediveligers and negligible shell growth of individuals. Larval shell growth was reduced significantly and abnormal at the lowest concentration tested (50 ng/l TBT). Ruiz *et al.* (1995b) reported that exposure to TBT caused abnormal larval development at 9%, 51% and 85% in the 125, 250, and 500 ng/l treatments, respectively. The mean length of the D-larvae produced in the 250 and 500 ng/l treatments was reduced compared to the D-larvae in the controls and at the lowest TBT treatment.

Langston (2020) noted that *Scrobicularia plana* numbers declined in heavily TBT contaminated areas during the 1980s when TBT concentrations peaked, e.g., the Solent area of the English Channel. Recruitment patterns and abundances recovered slowly over 25 years after the introduction of TBT regulatory measures. In the Solent area the sex ratios of *S. plana* were skewed towards males (2:1) where TBT sediment concentrations were highest. Langston (2020) noted that such masculinization of the clams by TBT probably reduced larval production and adversely affected reproduction in *S. plana*.

Therefore, the **worst-case resistance of *Scrobicularia plana* to TBT exposure is assessed as 'None'. Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'.**

Nine articles examined the effects of nanoparticulate metals on *Scrobicularia plana*. Eight of the articles reported sublethal effects, e.g., reduced burrowing activity, because of nanoparticulate metal exposure (Buffet *et al.*, 2011, 2012, 2013a&b, 2014; Pan *et al.* 2015; Bertrand *et al.*, 2016). However, Scola *et al.* (2021) reported that exposure to 500 µg/g copper nanoparticulates resulted in 100% mortality within 30 days of exposure. **Therefore, the worst-case resistance of *Scrobicularia plana* to nanoparticulate metals is assessed as 'None' but with 'Low' confidence** due to limited evidence of mortality. **Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'.**

5 Synthetic compounds – including Pesticides and Pharmaceuticals

A total of 81 results (ranked 'worst-case' mortalities) were obtained from 15 articles that examined the effects of 'Synthetic compounds' on the selected mollusc species. Pesticides/biocides were most studied (53% of the results), followed by 'Synthetics(other)' (21%), polychlorinated biphenyls (PCBs) (14%) and Pharmaceuticals (11% of the results) (Figure 5.1). *Cerastoderma edule* was the most studied species with 81% of the overall results (mainly under pesticides/biocides), followed by *Scrobicularia plana* with 15% of the results overall (Figure 5.2). *Macoma spp.* were only reported in a few studies of 'Pesticides/biocides' and represented only 4% of the results overall.

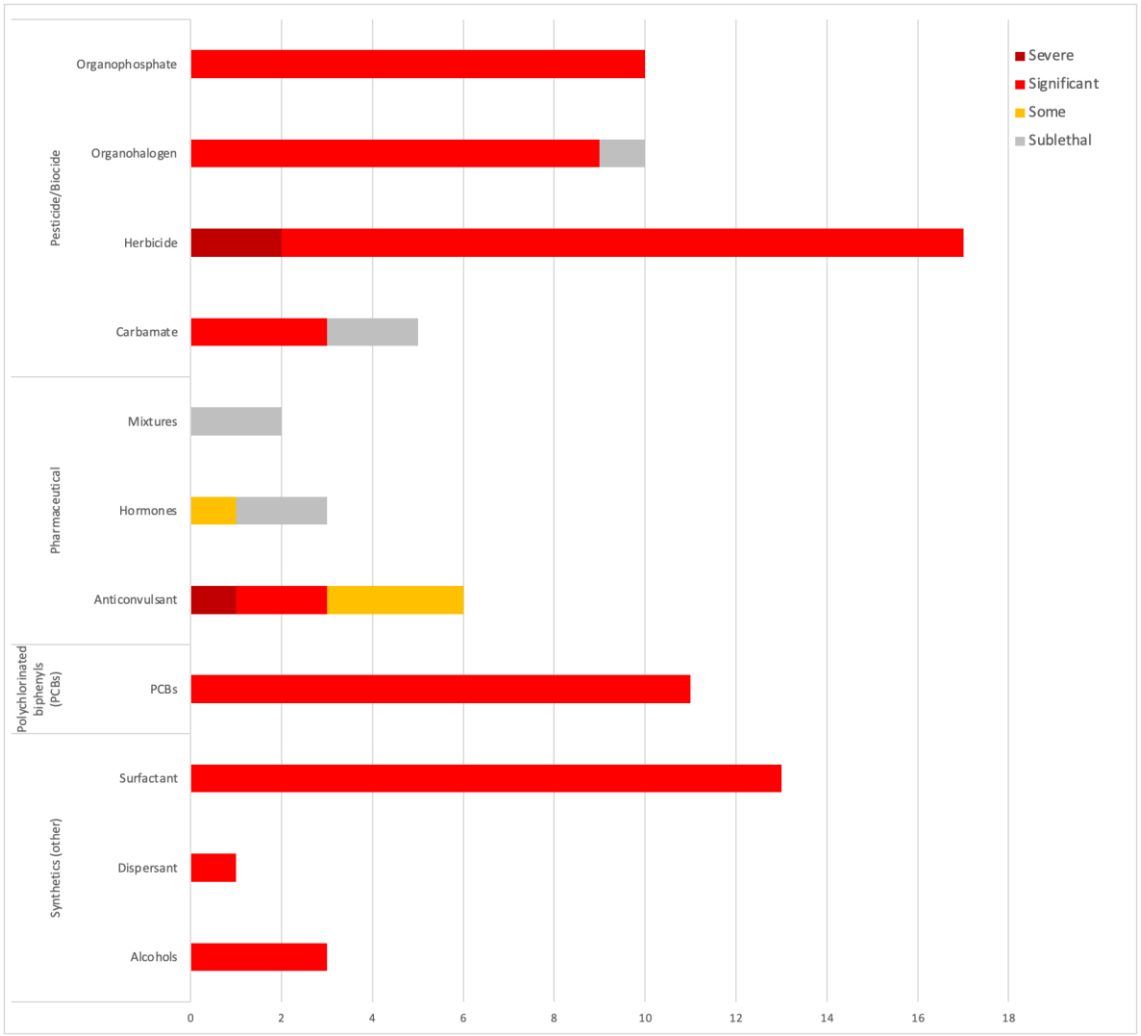

Figure 5.1. Count of worst-case ranked mortalities due to exposure to 'Synthetic compounds' in selected molluscs. Mortality is ranked as follows: 'Severe' (>75%), 'Significant' (25-75%), 'Some' (<25%), 'None' (no mortality reported), and 'Sublethal' effects.

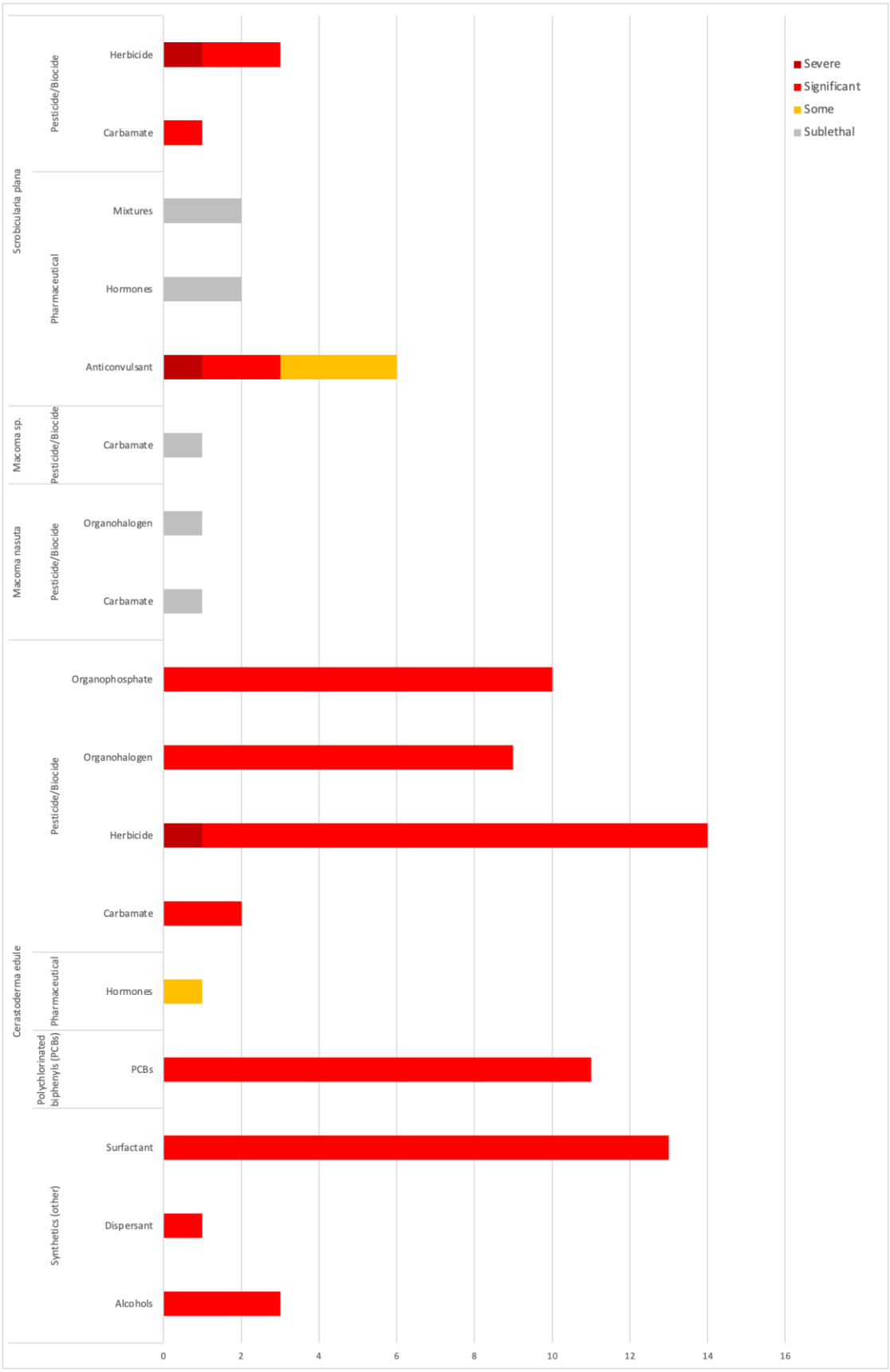

Figure 5.2. Count of worst-case ranked mortalities due to exposure to ‘Synthetic compounds’ in selected mollusc species. Mortality is ranked as follows: ‘Severe’ (>75%), ‘Significant’ (25-75%), ‘Some’ (<25%), ‘None’ (no mortality reported), and ‘Sublethal’ effects.

5.1 Pesticides/biocides

Where possible the pesticides/biocides were categorised by their function or target, for example herbicides, insecticides, rodenticides, or acaricides. A total of 42 results (worst-case ranked mortalities) were reported by the eight articles that examined the effects of pesticides/biocides on the selected mollusc species. The greatest proportion of the results (worst-case ranked mortalities) for the effects of pesticides/biocides were from studies of herbicides (40%), organohalogens (24%), organophosphates (24%), and carbamates (12%) (Figure 5.1). *Cerastoderma edule* dominated the studies, with 83% of the results, while studies of *Macoma* spp. contributed 7% of the results and *Scrobicularia plana* contributed 10% (Figure 5.2). Only 5% of the results reported 'Severe' mortality, but 88% reported 'significant' mortality, and 7% of the studies reported sublethal effects. The evidence is summarized below.

Akberali & Black (1980) investigated the behavioural responses and survival of the bivalve *Scrobicularia plana* in response to the insecticide Sevin. *Scrobicularia plana* were exposed to 1000, 5000, and 10,000 µg/l Sevin for 15 days to estimate the median lethal time. Significant mortality occurred in the two highest Sevin treatments with LT50s of 9 days at 10,000 µg/l and 15 days at 5,000 µg/l. No significant mortality occurred at 1,000 µg/l or in the control. For the behaviour tests, the clams were exposed to 1,000, 5,000, and 10,000 µg/l Sevin for 1 hour. The clams responded to Sevin exposure by closing their valve and reducing their heart rate.

Armstrong & Millemann (1974) investigated acute poisoning in the clam *Macoma nasuta* by insecticide exposure. Clams were exposed to Sevin at 15, 20, 25, and 30 mg/l for 96 hours, to assess behavioural changes. The inability of the clams to retract siphons or close valves was used as endpoints. After 96 hours of exposure around 50% of the clams had lost one or both siphons. The estimated 48-hour and 96-hour EC50s were 27.5 and 17 mg/l.

Dumbauld *et al.* (2001) observed the effects of the application of the pesticide Carbaryl on the estuarine benthic community in oyster culture sites. The small-scale experiment had four sets of replicate treatment and control plots at each of 2 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. No significant effects on *Macoma* spp. abundance were caused by pesticide exposure. The large-scale experiment was conducted over two years, two treatment sites were sprayed with 8.4 kg/ha Carbaryl, and the abundance of the benthic community was determined and

compared to control sites at 2 days, 51 days, 1 year and two years post-exposure. The average density of *Macoma* spp. was significantly different from the control plots at 51 days post-exposure but there were no significant differences at 2 days or 1 year.

Goncalves *et al.* (2016) investigated the effects of the herbicide Primextra® Gold TZ on the toxicity and fatty acid profile of the marine bivalves *Cerastoderma edule* and *Scrobicularia plana*. Two sizes classes (small and large) of bivalves were exposed to a range of concentrations between 0 to 60 mg/l Primextra® Gold TZ for 96 hours. Results showed that *Scrobicularia plana* was more sensitive to the herbicide than *Cerastoderma edule*. Calculated lethality values showed *Scrobicularia plana* of both size classes to be more sensitive to the herbicide than *Cerastoderma edule*, with LC50 values of 13.26 mg/l (L), 5.54 mg/l (S) and 28.78 mg/l (L), 27.25 mg/l (S), respectively. For both species, the larger individuals were more tolerant to herbicide exposure. For both species, 100% mortality occurred at high concentrations (but the concentrations were not specified).

Gutierrez *et al.* (2019) assessed the biomarker responses of the benthic clam *Scrobicularia plana* to the main active ingredients (S-metolachlor and Terbuthylazine) of the herbicide Primextra® Gold TZ. Clams of two sizes classes were exposed for 96 hours to a range of concentrations of each of the compounds (S-metolachlor- 2.048, 5.24, 13.42, 25.46, 30.5, 34.36, 39.40, 42.5, to 46.41 mg/L; Terbuthylazine - 40, 57.6, 69.2, 82.3, 95, 110.5, 125, and 138 mg/L). Both compounds were toxic to the clams, S-metolachlor was the most toxic with LC50s of 40.702 mg/l for large individuals and 41.517 mg/l for small, the LC50s for Terbuthylazine were 118.590 mg/l for large individuals and 108.418 mg/l for small.

Further studies by Portmann & Wilson (1971) and Portmann (1972) were not accessible but the data was provided by ECOTOX[7] and included in the 'Mollusc Evidence Summary' spreadsheet.

5.2 Pharmaceuticals

A total of 11 results (worst-case ranked mortalities) were reported by the seven articles that examined the effects of pharmaceuticals on the selected mollusc species. *Cerastoderma edule* were examined by one article yielding one result for 'Some' mortality and *Scrobicularia* accounted for the remaining results. No results on the impacts of pharmaceuticals on *Macoma balthica* were found in the evidence reviewed. 'Severe' mortality was reported in 9%

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

of the results, 18% reported ‘significant’, 36.5% ‘some’, and the remaining 36.5% only reported sublethal effects. The evidence is summarized below.

Almeida *et al.* (2017) investigated the toxicological impacts of Carbamazepine on *Scrobicularia plana*. The clams were exposed to a range of concentrations of Carbamazepine (0.00, 0.30, 3.00, 6.00, and 9.00 µg/l) for 96 hours. At each of the exposure concentrations, 17% mortality occurred, but 17% mortality also occurred in the control.

Almeida *et al.* (2017b) investigated the toxicity associated with the uptake and depuration of Carbamazepine in the clam *Scrobicularia plana*. Clams from two sampling sites (contaminated and non-contaminated) were exposed to environmentally realistic concentrations of Carbamazepine (0.00, 4.00, and 8.00 µg/l) for 14 days, followed by 14 days depuration period. There was no mortality in the clams exposed to Carbamazepine from the non-contaminated site. There was <7% mortality recorded in the clams exposed to Carbamazepine from the contaminated site.

Freitas *et al.* (2015) investigated the effects of the pharmaceutical Carbamazepine and low pH on the biochemical responses of the clam *Scrobicularia* plans. Clams from two sites (one pristine site and a mercury-contaminated site) were exposed to Carbamazepine at 3 µg/l for 96 hours at pH 7.8 and at pH 7.1. After 96 hours exposure, clams from the pristine site had no mortality in the Carbamazepine treatments at either pH. However, clams from the contaminated site had 11% mortality when exposed to carbamazepine at pH 7.8, but no mortality in the combined stressor treatment of low pH (7.1) and Carbamazepine exposure.

Freitas *et al.* (2015b) investigated the effects of Carbamazepine on macroinvertebrate species, comparing bivalves and polychaetes biochemical responses. The organisms were exposed to 0.3, 3, 6 and 9 µg/l Carbamazepine for 28 days in a renewal treatment. At the end of the experiment mortality of 10% was reported in the 0.3 µg/l treatment.

Freitas *et al.* (2016) investigated the effects of Carbamazepine and ocean acidification on the clam *Scrobicularia plana*. Clams were exposed for 28 days to the following treatments: Control (pH 7.8, without Carbamazepine), Carbamazepine exposure (3 µg/l, pH 7.8), low pH (pH 7.1, without Carbamazepine), combining stressors (pH 7.1 and carbamazepine 3.00 µg/l). After 28 days of exposure, 33% mortality had occurred in the Carbamazepine treatment and in the combined stressor treatment of Carbamazepine and low pH (pH 7.1). There was no mortality in the control treatment and 22% in the low pH exposure.

Langston *et al.* (2007) investigated the feminisation of male clams *Scrobicularia plana* by endocrine-disrupting chemicals through experimental exposures. The clams were exposed to spiked sediment mixtures of 17β-oestradiol (E2), 17α-ethinyloestradiol (EE2), octylphenol (OP) and nonylphenol (NP). The experiments were performed to establish whether exposure to known endocrine disrupting chemicals could cause changes in clam gonadal tissue. In the first experiment, clams were exposed to 17β-oestradiol (E2) or 17α-ethinyloestradiol (EE2) at sediment concentrations of 100 ug/kg wet weight, for six weeks. Experimental exposures showed oocytes from exposed clams were significantly larger than in the control. In the second experiment, adult clams were exposed to endocrine disrupting chemicals during an early 'window of sensitivity', starting in the winter months before differentiation and follicle development. The aim was to establish the reproductive effects of low- and high-level exposure to sediment-bound mixtures of E2, EE2, NP and OP throughout gonad development. In the low-level exposures, the clams were exposed to 100 E2 + 100 EE2 + 1000 NP + 1000 OP ug/kg (wet weight sediment) and in the high-level exposure, the clams were exposed to 1000 E2 + 1000 EE2 + 10,000 NP + 10,000 OP (ug/kg wet weight sediment) for a month. After the exposure treatments, one set of each of the duplicate tests were transplanted back to the estuary for four months, while the other set of tanks were maintained in the laboratory at conditions that matched those in the field. After the four-month period surviving clams were recovered and examined. In both low-level exposures (lab & transplant) 44% of males were observed to have ovotestis condition. In the high-level exposures recovery rates of the clams were too low for statistical analysis of intersex.

Lusher *et al.* (2017) investigated the effects of endocrine disrupting chemicals, bisphenol-A, and 17b-oestradiol, on *Cerastoderma edule*. Cockles were exposed to nominal concentrations of 17b-oestradiol (0.1 µg/l) and bisphenol-A (0.1 and 0.01 µg/l) for 60 days. At the end of the 60 days experiment, there were no statistical differences in the condition index of the cockles. However, there were lower survival rates of cockles exposed to both bisphenol-A (BPA) concentrations in comparison to 17b-oestradiol (E2) and the control, with mortality rates of 34, 31, 18, and 19%, respectively. There were no occurrences of intersex identified from exposure to 17b-oestradiol (E2) or bisphenol-A at the tested concentrations.

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. *Cerastoderma edule* was the only mollusc species examined

in those studies. Seventeen results were obtained from three studies that reported the effects of 'Synthetics (other)'. Maggi & Cossa (1973), Portmann & Wilson (1971) and Portmann (1972) were not accessible, but the data was provided by ECOTOX[8] and included in the 'Mollusc Evidence Summary' spreadsheet.

5.4 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) were examined in only two articles reviewed, producing 11 results. *Cerastoderma edule* was the only mollusc species examined in those studies. However, Portmann & Wilson (1971) and Portmann (1972) were not accessible, but the data was provided by ECOTOX and included in the 'Mollusc Evidence Summary' spreadsheet.

5.5 Sensitivity assessment – Synthetic compounds

The count of ranked mortalities due to 'Synthetic compounds' are summarized in Figure 5.1, Figure 5.2, and Table 5.1 below. The data presented in Table 5.1 include all life stages and articles where life stage was not reported or were unspecified (NR). 'Severe' or 'Significant' mortality was reported in 98% of the results on the effects of 'Synthetic compounds' on *Cerastoderma edule*, and 58% of *Scrobicularia plana* results, while only sublethal effects were reported in studies of *Macoma* spp.

5.5.1 *Cerastoderma* spp.

All three of the studies that examined exposure of *Cerastoderma edule* to 'Pesticides/biocides' reported 'severe' or 'significant' mortality. Goncalves *et al.* (2016) reported 96-hour LC50 values of 28.78 mg/l (large) and 27.25 mg/l (small) and 100% mortality at 60 mg/l in *Cerastoderma edule* exposed to the herbicide Primextra® Gold TZ. Portmann & Wilson (1971) and Portmann (1972) exposed *Cerastoderma edule* to a range of different 'Pesticides/biocides' (see Mollusc Evidence Summary). Overall, the evidence suggested that *Cerastoderma edule* was sensitive to all the 'Pesticides/biocides' tested. **Therefore, the worst-case resistance of *Cerastoderma edule* to 'Pesticides/biocides' is assessed as 'None'. Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'.** However, confidence in the assessment is 'Medium' due to the limited number of studies reviewed.

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

Table 5.1. Summary of count of worst-case ranked mortalities to 'Synthetic compound' contaminants reported in the evidence review and resultant proposed sensitivity assessments for mollusc 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
Cerastoderma edule											
	Pesticide/Biocide										
		Carbomate		**2**				2	L	M	M
		Herbicide	**1**	13				14	N	M	M
		Organohalogen		**9**				9	L	M	M
		Organophosphate		**10**				10	L	M	M
	Pharmaceutical										
		Hormones			**1**			1	M	H	L
	PCBs										
		PCBs		**11**				11	L	M	M
	Synthetics (other)										
		Alcohols		**3**				3	L	M	M
		Surfactant		**14**				13	L	M	M
Total			**1**	**62**	**1**			**64**			
***Macoma* spp.**											
	Pesticide/Biocide										
		Carbomate					**2**	2	H	H	NS
		Organohalogen					**1**	1	H	H	NS
Total							**3**	**3**	**H**	**H**	**NS**
Scrobicularia plana											
	Pesticide/Biocide										
		Carbomate		**1**				1	L	M	M
		Herbicide	**1**	2				3	N	M	M
	Pharmaceutical										
		Anticonvulsant		2	3	1		6	L	M	M
		Hormones					**2**	2	H	H	NS
		Mixture[9]					**2**	2	H	H	NS
Total			**1**	**5**	**3**	**1**	**4**	**14**	**N**	**M**	**M**
Total			**3**	**67**	**4**	**1**	**7**	**81**			

[9] 17β-oestradiol (E2), 17α-ethinyloestradiol (EE2), octylphenol (OP) and nonylphenol (NP)

Lusher *et al.* (2017) did not find any significant differences in the condition index, nor any evidence of intersex in *Cerastoderma edule* exposed to bisphenol-A and 17b-oestradiol. However, 17b-oestradiol exposure (at 0.1 µg/l for 60 days) did result in 'some' mortality compared to the control. Therefore, **the worst-case resistance of *Cerastoderma edule* to the human hormone 17b-oestradiol is assessed as 'Medium' but with 'Low' confidence due to the limited evidence. Hence, resilience is probably 'High' and sensitivity is assessed as 'Low'.**

Portmann & Wilson (1971) and Portmann (1972) exposed *Cerastoderma edule* to a range of different 'PCBs' (see Mollusc Evidence Summary). Their 48-hour LC50 ranged from 3 to 10 mg/l depending on the PCB. **Therefore, the worst-case resistance of *Cerastoderma edule* to 'PCBs' is assessed as 'Low'. Hence, resilience is assessed as 'Medium' and sensitivity as 'Medium'.** However, confidence in the assessment is 'Medium' due to the limited number of studies reviewed.

Maggi & Cossa (1973), Portmann & Wilson (1971) and Portmann (1972) examined the effects of a number of alcohols and surfactants on *Cerastoderma edule* adults and larvae. The articles were not accessible, but the data was provided by ECOTOX[10] and included in the 'Mollusc Evidence Summary' spreadsheet. Methanol exposure resulted in a 48-hour LC50 of 1,000 mg/l in adult *C. edule* while exposure to 2-Propen-1-ol resulted in a 48-hour LC50 of 100 mg/l in adult and larvae (Portmann & Wilson, 1971; Portmann, 1972). **Therefore, the worst-case resistance of *Cerastoderma edule* to the alcohols tested is assessed as 'Low', resilience as 'Medium' and sensitivity as 'Medium'.** However, it should be noted that the concentrations used were high and the confidence in the assessment is 'Low'.

Maggi & Cossa (1973), Portmann & Wilson (1971), and Portmann (1972) also derived lethal doses (LD50s) and LC50s for a number of surfactants. Therefore, **the worst-case resistance of *Cerastoderma edule* to the surfactants tested is assessed as 'Low', resilience as 'Medium' and sensitivity as 'Medium'.** However, the confidence in the assessment is 'Low' due to the limited evidence.

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

5.5.2 *Macoma* spp.

Three articles examined the effects of 'Pesticides/biocides' on *Macoma* spp. Armstrong & Millemann (1974) exposed *Macoma nasuta* to Sevin at 15, 20, 25, and 30 mg/l for 96 hours. After 96 hours of exposure, around 50% of the clams had withdrawn one or both siphons, with estimated 48-hour and 96-hour EC50s of 27.5 and 17 mg/l Sevin, respectively. But no mortality was reported. Boese *et al.* (1990) reported sublethal effects on the physiology of *Macoma* spp. exposed to 5.2 – 7.8 µg/l hexachlorobenzene for 3-7 days. Dumbauld *et al.* (2001) examined the effects of pesticide treatment of oyster sites on benthic infauna. No significant effects on *Macoma* spp. abundance were observed 24 hours, 2 weeks, 1 month and 1 year after the application of Carbaryl at 5.6 kg/ha. The average density of *Macoma* spp. was significantly different from the control plots at 51 days post-exposure to 8.4 kg/ha Carbaryl but there were no significant differences after 2 days or 1 year (Dumbauld *et al.*, 2001). Therefore, **the worst-case sensitivity of *Macoma* spp. to the 'Pesticides/biocides' tested is assessed as 'Not sensitive'** since only sublethal or transient effects were reported. However, confidence in the assessment is 'Low' due to the limited number of studies and pesticides tested.

5.5.3 *Scrobicularia* spp.

Three articles examined the effects of 'Pesticides/biocides' on *Scrobicularia plana*. Akberali & Black (1980) reported significant mortality in *S. plana* exposed to the highest concentration of Sevin (a carbamate) with LT50s of 9 days at 10,000 µg/l and 15 days at 5,000 µg/l. No significant mortality occurred at 1,000 µg/l or in the control. Goncalves *et al.* (2016) reported that *Scrobicularia plana* of both size classes were more sensitive to the herbicide Primextra® Gold TZ than *Cerastoderma edule*, with 96-hour LC50 values of 13.26 mg/l (L), 5.54 mg/l (S) and 100% mortality at 60 mg/l. Gutiérrez *et al.* (2019) reported that S-metolachlor was the most toxic to *S. plana* with 96-hour LC50s of 40.702 mg/l for large individuals and 41.517 mg/l for small, and 96-hour LC50s for Terbuthylazine of 118.59 mg/l for large individuals and 108.42 mg/l for small. **Therefore, the worst-case resistance of *Scrobicularia plana* to the pesticides tested is assessed as 'None', resilience as 'Medium' and sensitivity as 'Medium'.**

Six articles examined the effects of 'Pharmaceuticals' on *Scrobicularia plana*. Almeida *et al.* (2017b) examined the effect of the anticonvulsant Carbamazepine on *S. plana* but did not detect significant effects. Freitas *et al.* (2015) reported 11% mortality in *S. plana* from a mercury contaminated site when exposed to 3 µg/l Carbamazepine at pH 7.8. Freitas *et al.*

(2015b) reported 10% mortality exposed to Carbamazepine 0.3 µg/l for 28 days. Freitas *et al.* (2016) reported 33% mortality of *S. plana* after 28 days in 3 µg/l Carbamazepine treatment and in the 3 µg/l Carbamazepine and low pH (pH 7.1) treatment. There was no mortality in the control treatment but 22% mortality in the low pH exposure. **Therefore, the worst-case resistance of *Scrobicularia plana* to the anticonvulsant Carbamazepine is assessed as 'Low', resilience as 'Medium' and sensitivity as 'Medium'** but with 'Low' confidence due to the limited evidence.

Langston *et al.* (2007) examined the effects of endocrine disrupting chemicals (EDCs) on *Scrobicularia plana*. Langston *et al.* (2007) examined the effect of the human hormone 17α-ethinyloestradiol (EE2) or 17β-oestradiol (E2) alone, and in mixtures with other EDCs, that is 17β-oestradiol (E2), 17α-ethinyloestradiol (EE2), octylphenol (OP) and nonylphenol (NP) in sediment for six weeks. In low-level exposures, 44% of males were observed to have ovotestis condition. In the high-level exposures, recovery rates of the clams were too low for statistical analysis of intersex. **Therefore, the sensitivity of *Scrobicularia plana* to the endocrine disrupting chemicals tested is assessed as 'Not sensitive' because only sublethal (intersex) effects were reported.** Langston (2020) reported that this intersex (feminisation of male clams) was widespread in the southwest of England, with 60% of males from the Bristol Channel and Severn Estuary exhibiting the condition, although the male to female sex ratio was ca 1:1. Some of the highest levels of intersex were observed in the Mersey Estuary, which is highly industrialised and reported to exhibit elevated EDCs in flounders (Langston, 2020). However, the implications for fecundity and the population are unknown (Langston, 2020).

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6 Other substances

'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. The relevant evidence is summarized below.

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

Species	Type	Contaminant	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Cerastoderma edule											
	Inorganic chemicals										
		Nitric acid		**1**				1	L	M	M
		Potassium cyanide		**1**				1	L	M	M
		Sodium chloride		**1**				1	L	M	M
		Sodium hydroxide		**1**				1	L	M	M
		Sodium thiocyanate		**1**				1	L	M	M
		Sulphuric acid		**1**				1	L	M	M
		Chlorine oxide		**1**				1	L	M	M
	Other										
		Dermol		**1**				1	L	M	M
	Paint										
		Historic paint particles				**1**		1	H	H	NS
		Modern paint particles	**1**					1	N	M	M
		Silicone paint particles				**1**		1	H	H	NS
	Mixtures										
		Polluted site					**2**	2	H	H	NS
		Pulverized fuel ash		**2**				2	N	M	M
		Wastewater					**1**	1	H	H	NS
***Macoma* spp.**											
	Inorganic chemicals										
		Bromate	**1**						N	M	M
		Hydrogen sulphide		**1**				1	L	H	L
	Mixtures										
		Pulverized fuel ash			**1**			1	M	H	L
		Wastewater					**1**	1	H	H	NS

Species	Type	Contaminant	Severe	Significant	Some	None	Sublethal	Total	Resistance	Resilience	Sensitivity
Scrobicularia plana											
	Mixtures										
		Mine spill							L	M	M
		Fish farm effluents					**3**	3	H	H	NS
		Wastewater					**1**	1	H	H	NS
Total			**3**	**11**	**1**	**2**	**8**	**24**			

6.1 *Cerastoderma* spp.

Four articles examined the effects of a number of 'Other substances' on *Cerastoderma edule*.

6.1.1 Inorganic chemicals

Portmann & Wilson (1971) examined the effect of a number of inorganic chemicals on *Cerastoderma edule*. The 48-hour LC50s were as follows: 330 to 1,000 mg/l nitric acid; 25 mg/l potassium cyanide; 66,000 mg/l sodium chloride; 330 to 1000 mg/l sodium hydroxide; of >500 mg/l sodium thiocyanate; >500 mg/l chlorine dioxide (ClO_2), and 200 to 500 mg/l sulphuric acid. **Therefore, the worst-case resistance would be assessed as 'Low', so that resilience is probably 'Medium' and sensitivity 'Medium'. However, confidence is 'Low'** since the evidence is based on a single study and the concentrations used are high. For example, GESAMP (2019) criteria describe EC/LC50 values of >100 – ≤1000 mg/l as 'Practically non-toxic'.

6.1.2 Paint particles

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 *Cerastoderma edule* 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.

6.1.3 Mixtures

'Mixtures' include articles that have assessed the impacts of wastewater discharge, pulverized fuel ash, industrial effluent, fish farm effluents and polluted sites on the selected molluscs. The results are tabulated in Table 6.1

Bergayou *et al.* (2019) observed the changes in an estuarine ecosystem following the cessation of wastewater discharge. Three campaigns were undertaken, two were carried out while the estuary was receiving wastewater discharge in 2001 and 2002, and one campaign was carried out after the cessation of the pollution in 2003. When the ecosystem was receiving wastewater discharge, the intertidal macrobenthic fauna composition was similar, with *Hydrobia ulvae* as the dominant species, followed by *Hediste diversicolor* and *Scrobicularia plana* in decreasing order. After the termination of wastewater discharge, the number of individuals was significantly higher than in the period when the ecosystem received wastewater. In 2003, the dominance of species was *Hediste diversicolor*, followed by *Hydrobia ulvae*, *Cerastoderma edule*, and *Scrobicularia plana* in decreasing order. However, both before and after the end of wastewater discharge, the Phylum Mollusca was dominant, followed by Annelids and Crustaceans. The species richness was higher in 2003 following the end of the wastewater discharges, with 22 species instead of 14. The total abundance percentage of *Cerastoderma edule* increased from <4% in 2001 and 2002 to 23% in 2003.

Bowmer *et al.* (1994) investigated the effects of Pulverized Fuel Ash (PFA) exposure on the cockle *Cerastoderma edule* for 3 and 9 months. The impacts on survival, growth, metal

accumulation, and histology were investigated using model ecosystem experiments. The cockles were exposed to 100% PFA or 50% PFA. After 230 days, the mortality rate of the 100% PFA treatment was 43%, and the mortality rate of the 50% PFA treatment was 42.5%. The growth of the cockles exposed to PFA treatments was notably reduced compared to the control.

Jenner & Bowmer (1990) investigated the effects of Pulverized Fuel Ash (PFA) exposure on Arenicola marina, *Cerastoderma edule*, and *Macoma balthica*. The impacts on survival and metal accumulation were investigated through 90-day mesocosms experiments. The test organisms were exposed to 100% PFA, 50% PFA or dosed daily with 500 ml PFA. After 90 days, the mortality rate of *Cerastoderma edule* at 100% PFA treatment was 43.3%, the mortality at 50% PFA was 31.7% and the mortality in the dosed treatment was 70.6%. The control mortality was 26%. In *Macoma balthica*, mortality could not be followed in three of the treatment tanks because a substantial wild population of *M. balthica* still survived in all mesocosms. However, there was 20% mortality reported in the 100% PFA treatment.

Savari *et al.* (1991) investigated the physiological state of the cockle *Cerastoderma* from different sites in Southampton Water. Cockles of different age classes were collected from seven sites on monthly basis throughout 1985 and part of 1986 to establish the condition index and growth rates. The physiological characteristics (clearance rate, assimilation rate, oxygen consumption, ammonia excretion rate) of the cockles from two sites (relatively unpolluted and heavily polluted) were measured in the field during the summer of 1987 to determine the scope of growth. The results showed measurable differences in the condition index of the cockles from different sites, which broadly related to the level of contamination. In addition, cockles from the relatively unpolluted site had better metabolic status than those from the heavily polluted site. They reported a direct correlation between tissue metal level and scope for growth based on laboratory exposure to copper at concentrations from 3 to 40 µg/l.

Overall, wastewater discharge was shown to reduce the abundance of *Cerastoderma edule* in the affected area (Bergayou *et al.*, 2019). Exposure to pulverised fuel ash was reported to result in ‘Severe’ mortality in *Cerastoderma edule* (Jenner & Bowmer, 1990; Bowmer *et al.*, 1994), while pollution in Southampton Water resulted in reduced scope for growth (Savari *et al.*, 1991). However, the exact nature of the contaminants in each mixture is unclear. **Therefore, the worst-case resistance of *Cerastoderma edule* to pulverised fuel ash exposure is assessed as ‘None’, resilience as ‘Medium’ and sensitivity as ‘Medium’ but**

with 'Low' confidence. However, its **resistance to wastewater discharge may be 'Medium' and its sensitivity 'Low' depending on the nature of the contaminants involved** but confidence is 'Low' due to the lack of evidence.

6.2 *Macoma* spp.

Five articles examined the effects of 'Other substances' on *Macoma* spp.

Caldwell *et al.* (1975) exposed *Macoma balthica* to 100, 330, 1000, 3,300, and 10,000 µg/l hydrogen sulphide for 96 hours. The longer the clams were exposed to hydrogen sulphide the lower the concentration was required to cause 50% mortality. The LC50 at 24, 48, and 96 hours were 10,000, 8,000 and 6,000 µg/l, respectively. Crecelius (1979) examined the effect of bromate on *Macoma inquinata* and reported 100% mortality after 72 hours at 880 mg/l bromate. **Therefore, the worst-case resistance of *Macoma* spp. to the inorganic chemicals tested is assessed as 'None', resilience as 'Medium' and sensitivity as 'Medium' but with 'Low confidence due to the limited evidence.**

Wastewater discharge was shown to reduce the abundance of *Macoma cumana* in the affected area (Bergayou *et al.*, 2019). Exposure to pulverised fuel ash was reported to result in 20% mortality of *Macoma balthica* in the 100% PFA treatment (Jenner & Bowmer, 1990). **Therefore, the worst-case resistance of *Macoma balthica* to pulverised fuel ash exposure is assessed as 'Medium', resilience as 'High', and sensitivity as 'Low' but with 'Low' confidence.** However, its **resistance to wastewater discharge may be 'Medium' and its sensitivity 'Low' depending on the nature of the contaminants involved** but confidence is 'Low' due to the lack of evidence.

6.3 *Scrobicularia* spp.

Four articles the effects of mixtures on *Scrobicularia plana*. Lopes *et al.* (2014) is included under 'Metals' above.

Rida *et al.* (2004) investigated the lethal effects of sediment from the Guadalquivir estuary following the Aznalcollar mining spill. Toxicity tests using dilutions (0.3, 1.8, 7.9, 20, and 32%) of toxic mud and sediment from environmental stations were tested using the amphipod *Ampelisca brevicornis* and the clam *Scrobicularia plana*. Two bioassays were performed to assess the toxicity of the mud dilutions (96-hour and 10-day). The results showed the amphipods to be more sensitive to the spill than the clams. At dilutions of toxic mud above 1.8%, there was 100% mortality of amphipods. For the clams, no mortalities were observed

at toxic mud dilutions of 0.3% and 1.8% within the first 72 hours and by the end of the bioassay (96 hours) there were no significant differences in mortality to the environmental stations. However, at dilutions above 1.8% significant mortality occurred. At the dilution of 20% and 32% toxic mud, 100% mortality of clams occurred by the end of the 96-hour exposure. At the dilution of 7.9%, mortality of around 75% occurred by the end of the 96-hour bioassay. The 96-hour LC50 of the toxic mud to the clams was calculated at 3.25%.

Silva *et al.* (2012) investigated the effects of fish farm effluents on benthic community structure and biomarker responses of the clam *Scrobicularia plana*. The benthic fauna and clam samples were collected from five sites following a gradient of contamination from the aquaculture effluent to the control site. The numbers of species, abundance, richness, and Shannon diversity were the biodiversity indicators calculated for each sampling site. The morphological and reproduction status of clams were assessed using the condition factor and gonadosomatic index, respectively. Benthic biodiversity indicators were negatively correlated significantly with organic matter. The condition factor was significantly increased at sites nearest to the fish farm effluent compared to the control. The gonadosomatic index was positively correlated significantly with the distance to fish farm effluent and negatively correlated with organic matter.

Overall, wastewater discharge was shown to reduce the abundance of *Scrobicularia plana* in the affected area (Bergayou *et al.*, 2019), while Silva *et al.* (2012) reported changes in its condition. However, Rida *et al.* (2004) reported that the exposure to toxic mud from a mine spill resulted in ‘Significant’ mortality of *Scrobicularia plana* at dilutions above 1.8%; the 96-hour LC50 of the toxic mud to the clams was calculated at 3.25%. However, the exact nature of the contaminants in each mixture is unclear. **Therefore, the worst-case resistance of *Scrobicularia plana* to mine spill effluent exposure is assessed as ‘Low’, resilience as ‘Medium’ and sensitivity as ‘Medium’ but with ‘Low’ confidence.** However, its **resistance to wastewater discharge may be ‘Medium’ and its sensitivity ‘Low’ depending on the nature of the contaminants involved** but confidence is ‘Low’ due to the lack of evidence.

7 Conclusions

This report presents the finding of a time limited Rapid Evidence Assessment (REA) of the effects of contaminants on selected molluscs. The literature review focused on *Cerastoderma* spp., *Macoma* spp. (plus the syn. *Limecola balthica*) and *Scrobicularia* spp.. The key findings are summarized below.

- A total of 264 results were obtained from 82 articles that studied the effect of contaminants on the selected mollusc species.
- The articles reviewed reported mortality ('Severe' to 'Some') in 78% of results (worst-case ranked mortalities), no mortality ('none') in 8.7% of results, and sublethal effects in 13% of results.
- 'Transitional metals' was the most studied contaminant group and contributed *35%* of the results in the review. 'Hydrocarbons (Petrochemical)' dispersant and oil mixtures contributed 26% of the results and 'Pesticides/biocides' 15% of the results from the evidence review.
- *Cerastoderma* spp. was examined in 24 (29%) of the studies reviewed and contributed 62% of the results in the review to the widest range of contaminant types). In comparison, *Scrobicularia* spp. was examined in 32 (39%) of the studies and contributed 19% of the results and *Macoma* spp. was examined in 34 (41%) of the studies but contributed 18% of the results
- '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.
- Dispersed oils, dispersant: oil mixtures and dispersants were the next most toxic group of contaminants in terms of in terms of mortality, followed by 'Pesticides/biocides'.
- 'Pharmaceuticals' were reported to result in mortality and sublethal effects in almost equal number of results. The reported effects varied between the pharmaceutical tested and the species examined.
- Exposure to human hormones and other endocrine disruptor chemicals were shown to cause intersex (feminisation of males) in *Scrobicularia plana* but the effects on

reproduction and population dynamics remains unclear (Langston, 2020). The human hormone 17b-oestradiol was reported to result in ‘Some’ mortality in *Cerastoderma edule* but not intersex.

- The volume of evidence (in terms of number of relevant articles found) was surprisingly low, except for the commercially harvested species, *Cerastoderma edule*. And in most cases, except ‘Transitional metals’ the effects of any one contaminant on *Macoma* spp. or *Scrobicularia* spp. 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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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[11], 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.

[11] 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 was reported but not quantified or no detail was 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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