



Review

Toxicity of seven priority hazardous and noxious substances (HNSs) to marine organisms: Current status, knowledge gaps and recommendations for future research



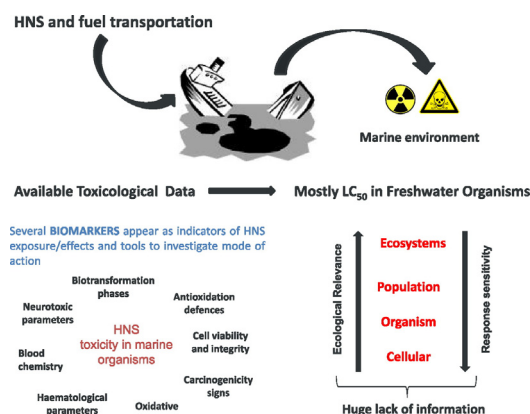
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HIGHLIGHTS

- Review of toxicological effects of seven HNS towards aquatic species
- Lack of information for marine species but HNS toxicity was found for some organisms
- Studies of long-term effects of the selected HNS in marine species are required
- Biochemical biomarkers are useful tools for studying HNS toxicity
- Using realistic HNS exposure scenarios will improve protection of marine species

GRAPHICAL ABSTRACT



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ABSTRACT

Shipping industry and seaborne trade have rapidly increased over the last fifty years, mainly due to the continuous increasing demand for chemicals and fuels. Consequently, despite current regulations, the occurrence of accidental spills poses an important risk. Hazardous and noxious substances (HNSs) have been raising major concern among environmental managers and scientific community for their heterogeneity, hazardous potential towards aquatic organisms and associated social-economic impacts. A literature review on ecotoxicological hazards to aquatic organisms was conducted for seven HNSs: acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene. Information on the mechanisms of action of the selected HNS was also reviewed. The main purpose was to identify: i) knowledge gaps in need of being addressed in future research; and ii) a set of possible biomarkers suitable for ecotoxicological assessment and monitoring in both estuarine and marine systems.

Main gaps found concern the scarcity of information available on ecotoxicological effects of HNS towards marine species and their poorly understood mode of action in wildlife. Differences were found between the sensitivity of freshwater and seawater organisms, so endpoints produced in the former may not be straightforwardly

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employed in evaluations for the marine environment. The relationship between sub-individual effects and higher level detrimental alterations (e.g. behavioural, morphological, reproductive effects and mortality) are not fully understood. In this context, a set of biomarkers associated to neurotoxicity, detoxification and anti-oxidant defences is suggested as potential indicators of toxic exposure/effects of HNS in marine organisms. Overall, to support the development of contingency plans and the establishment of environmental safety thresholds, it will be necessary to undertake targeted research on HNS ecotoxicity in the marine environment. Research should address these issues under more realistic exposure scenarios reflecting the prevailing spatial and temporal variability in ecological and environmental conditions.

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1. Introduction

The continuous increasing demand for chemicals and fuels, used in a variety of applications, industries, and consumer products led to a rapid growth of shipping industry and seaborne trade over the last fifty years. Maritime trade and transportation of substances are in fact one of the foundations of global economy, covering nowadays more than 90% of global trade (MKC, 2012). Despite the technological advances and increased efficiency of transportation processes, shipping is still one of the most dangerous industries to the environment (Parfomak and Frittelli, 2005). This is mainly due to leakage of hazardous substances either from routine operations (e.g. loading, discharging and bunkering) or discharges from ships and to accidental spills of goods, chemicals and fuels into the sea in consequence of shipping accidents (e.g. collisions, grounding) (MKC, 2012).

In the field of marine pollution, attention has been generally drawn to oil and fuel spills. These have great visual impact, and immediate consequences to ecosystems and economic activities. Nevertheless, the occurrence of spills of other chemical substances has increased over the last decades as a consequence of increased shipping (MKC, 2012). A good example is that of hazardous and noxious substances (HNSs) defined as “substances other than oil, which, if introduced into the marine environment, have the potential to create hazards to human health, to harm living resources and marine life, to damage amenities or to interfere with other legitimate uses of the sea” (CEDRE, 2012). HNS can be more toxic than oils and their hazardousness can be more wide reaching (ITOPF, 2011). Hence, there is a need to better understand the environmental fate and implications of these substances to support the development of strong and consistent contingency plans (MKC, 2012). However, a wide variety of chemicals exhibiting different physical and chemical properties fall into the HNS category. Thus, as a consequence of these different properties, HNS spill contingency protocols cannot be as straightforward as those adopted for oil spills since these chemicals can have an assortment of possible behaviours/interactions and of potential effects on flora, fauna and human health when released into the environment (ITOPF, 2011). There is a need to better understand the environmental fate and implications of these

substances to support the pre-planning of risk management contingency protocols and regulation of HNS transport (CEFAS, 2009).

A literature review on ecotoxicological hazards to aquatic organisms was conducted for seven HNS, viz. acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene [the three isomers: meta- (m), para- (p) and ortho- (o)]. These target HNS were selected, from a priority list established (Neuparth et al., 2011b), for their different physico-chemical properties, toxicity for living organisms and frequency of transportation. The main purpose is to identify information and knowledge gaps in need of being addressed for hazard identification and ecological risk assessment (ERA). Information on the mechanisms of action of the selected HNS was collated to gain further understanding on their toxicity and to identify a set of possible biomarkers suitable for ecotoxicological assessment and monitoring in estuarine and coastal systems.

2. Material and methods

This review is based on studies identified from 1990 onward on the seven selected HNSs. The aim was to obtain information on toxicity of selected HNS, as well as on statistical endpoints, i.e., median lethal concentration (LC₅₀), effective concentration at x% (EC_x), concentration causing x% inhibition of the response observed (IC_x), as well as no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). Searches were conducted in various information sources: i) Scopus (<http://www.scopus.com/>); ii) ISI Web of Knowledge (WoK, <http://wokinfo.com/>) databases; iii) ECOTOXicology database from Environmental Protection Agency (EPA); iv), datasheets from European Union reports; v) documentation from the Organisation for Economic Co-operation and Development (OECD); vi) documentation from the Agency for Toxic Substances and Disease Registry (ATSDR); and vii) documentation from the International Agency for Research on Cancer (IARC). Scopus and WoK provided most of the significant literature. Several keywords and combinations of search terms were used: “name of HNS of interest” or “chemical synonym” (acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene) in combination with one or more of the following

terms “AND toxicology”, “AND aquatic organisms OR marine organisms OR freshwater organisms”, “AND LC₅₀”, “AND spills”, “AND biomarkers”, “AND mode/mechanisms of action”, “AND neurotoxicity”, “AND metabolism”. Criteria for inclusion in the review were related to the detail provided by the studies (e.g. species and age of test-organisms, duration of the test, the most relevant exposure conditions (e.g. static, renewal or flow-through system; temperature and water kind) and measured end-points) and authors' awareness and control of essential experimental conditions that may bias the test results. Compliance with standardised guidelines (OECD, ASTM) or conditions described within these were used as quality criteria. Given the scarcity of studies using coastal or marine species, data on freshwater species were also included. For each HNS of interest, the data was arranged according to trophic level. With regard to the mechanisms of toxicity and modes of action of HNS, studies using mammalian species were also reviewed. Physico-chemical parameters of each HNS were obtained from the reviewed literature, ASTDR, OECD and IARC reports, safety datasheets and chemical sites (e.g. PubChem, <https://pubchem.ncbi.nlm.nih.gov/>); ChemIDplus, <http://chem.sis.nlm.nih.gov/chemidplus/>).

3. Physico-chemical properties

After a spill, diffusion and effects of HNS on the marine environment will depend upon a number of factors, such as, meteorological conditions, local topography and the quantity of chemical released. Nonetheless, the environmental fate and toxicity of a chemical are dictated by its physico-chemical properties (ITOPF, 2011). In the case of oils, their behaviour is easily predicted as the majority of them float on sea surface and are immiscible with water (CEFAS, 2009). On the contrary, HNS can exhibit multiple behaviours: substances can dissolve, evaporate, float or sink in consequence of their different properties (Neuparth et al., 2011b). Because of that, prediction of possible deleterious effects on ecosystems and the development of contingency responses to HNS incidents are not as straightforward as for oils. For this reason, thorough knowledge on the physico-chemical and toxicological properties of the chemical in question is more required than ever (Cunha et al., 2014).

Table 1 summarises the physico-chemical characteristics of acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene, which determine their behaviour, distribution and persistence in the environment. All these HNSs are volatile hydrocarbon derivatives and are included in the priority list drawn up by Neuparth et al. (2011b). Diversity in terms of physico-chemical features is generally exhibited, e.g. some differences in melting and boiling points, density, vapour pressure, soil organic carbon–water partition coefficient ($\log K_{oc}$), octanol – water partition coefficient ($\log K_{ow}$) and solubility (Table 1). A common behaviour with regard to bioconcentration is however observed. Published bioconcentration factors (BCF) show that all HNSs considered in this study present a low potential to bioaccumulate in aquatic organisms (Table 1), considering the threshold of $BCF \geq 2000$ to classify a substance as bioaccumulative (REACH, 2015). Despite the generally low BCF values, there is sound evidence in the reviewed literature that these HNSs show high acute toxicity and potential chronic toxicity towards aquatic organisms (see below). Thus, the hazard of these HNSs to marine ecosystems is more likely to be a direct effect of the pollutant on a particular species or group of species rather than their transfer through food chain.

4. General toxicity to aquatic organisms

Apart from environmental conditions, the impact of a chemical introduced in the marine environment depends upon its toxicity, the length of time during which organisms are exposed and the sensitivity of the organisms to a particular chemical (ITOPF, 2011). In the case of HNS, knowledge on their effects on marine organisms is quite limited in comparison to oils and polycyclic aromatic hydrocarbons (PAHs)

(McGowan et al., 2013), since most of ecotoxicological experiments have been conducted on freshwater organisms. Effects of these HNSs to micro- and macro-algae, aquatic plants and invertebrates, as well as vertebrates, are compiled in Tables 2 and 3, respectively. Overall, our literature review led to the identification of 121 ecotoxicological bioassays carried out with the HNS of interest. A summary list of the aquatic organisms used in these bioassays is shown in Table 4. In total, 68 aquatic species were employed as test organisms.

The analysis of the compiled data indicated that there are important information gaps limiting hazard assessment and mode of action of the HNS in question. It is clear that the knowledge available for marine organisms is considerably scarce: 75% of the species used in the bioassays were freshwater organisms against a small percentage (25%) belonging to marine systems (Table 4). Understanding toxicological effects on freshwater organisms may serve as a basis for predicting possible responses of marine organisms to HNS contamination. Nevertheless, freshwater and marine organisms inhabit environments with severe differences (e.g. salinity), having developed physiological adaptations to cope with their habitat's characteristics (Schmidt-Nielsen, 1997). Salinity, for example, influences the distribution, abundance and physiology of aquatic species. It may also influence synergically and antagonistically the toxicity of several substances due to speciation, altered bioavailability and physiological phenomena (Heugens et al., 2001; Rodrigues et al., 2014). Consequently, the effects of HNS on marine organisms can differ from those triggered in freshwater organisms. For instance, exposure to hexane or xylene, under similar experimental conditions, of rotifer *Brachionus calyciflorus* (freshwater) and *Brachionus plicatilis* (seawater) suggests that the marine species is more tolerant to those HNSs than the freshwater species (Snell et al., 1991a, b), showing considerably higher LC₅₀ (hexane: 68 mg/L and 154 mg/L for *B. calyciflorus* and *B. plicatilis*, respectively; xylene: 258 mg/L and 496 mg/L for *B. calyciflorus* and *B. plicatilis*, respectively) (Table 2). The reliance on data solely from freshwater organisms can lead to inaccurate derivation of environmental risk limits and the development of either overprotective or insufficiently appropriate prevention/contingency procedures.

As to the duration of the exposure, short exposure periods (24 h–96 h) were generally tested for the majority of HNS. Data evidently demonstrate the paucity of knowledge about the effects that longer exposure periods to acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene may trigger in marine and coastal organisms: 74% of the reviewed literature refers to acute exposures whereas in only 26% of the cases have longer exposure periods (> 8 days) been assessed (Tables 2 and 3). Considering their physico-chemical characteristics, especially of those classified as evaporators according to the European Classification System for chemicals (Table 1), the concentration of HNS in the marine compartment would be expected to quickly diminish with time possibly reaching environmental or undetectable levels (Whittier et al., 2005; Annable et al., 2008). Given the heterogeneous nature of HNS, this process may range from a few days (e.g. acrylonitrile with mean half-life time ($t_{1/2}$) in water of 170 h (Long et al., 2002) to a few years (e.g. the very slow hydrolysis of n-butyl acrylate with mean $t_{1/2}$ of 1100 days) (OECD-SIDS, 2002a). However, the effects induced by long exposure periods are still barely understood. Also, in the event of a HNS spill, some organisms may eventually move into cleaner areas while others with a sedentary lifestyle will remain in the contaminated area. Studies employing more realistic exposure conditions are thus required to gather important information on potential adverse outcomes elicited by such transient exposures to HNS.

A broad range of aquatic testing models – bacteria, ciliates, algae, plants, rotifers, annelids, molluscs, crustaceans, echinoids, amphibians and fish – has been used to evaluate the ecotoxicity of the HNS in question. Data analysis clearly demonstrates that the ecotoxicological effects of acrylonitrile, trichloroethylene and xylene are better characterised for an assortment of aquatic organisms than those of n-butyl acrylate, cyclohexylbenzene, hexane and isononanol for which only a few studies

Table 1
Physico-chemical properties of acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene and information about their anthropogenic use, associated hazards and persistence in aquatic environment.

Hazardous and noxious substances							
	Acrylonitrile ^a	N-butyl acrylate ^b	Cyclohexylbenzene ^c	Hexane ^d	Isononanol ^e	Trichloroethylene ^f	Xylene ^g
CAS number	107-13-1	141-32-2	827-52-1	110-54-3	3452-97-9	79-01-6	1330-20-7 (mixed isomers) 108-38-3 (<i>m</i> -xylene) 95-47-6 (<i>o</i> -xylene) 106-42-3 (<i>p</i> -xylene)
Chemical formula	C ₃ H ₃ N	C ₇ H ₁₂ O ₂	C ₁₂ H ₁₆	C ₆ H ₁₄	C ₉ H ₂₀ O	C ₂ HCl ₃	C ₆ H ₄ (CH ₃) ₂ Contain three isomers (meta-, ortho-, and para-xylene)
Physical appearance (P atm, 20 °C)	Colourless to pale yellow volatile liquid with a pungent odour	Clear colourless liquid with a fruity and sharp odour	Colourless oily and odourless liquid	Colourless and clear volatile liquid with faint odour	Colourless oily liquid with alcoholic odour	Non-flammable, colourless liquid with a somewhat sweet odour	Colourless, sweet-smelling and highly flammable liquid
Melting point	−83.5 °C	−64 °C	5–7 °C	−95.35 °C	−80 °C	−84.8 °C	−47.8–13.2 °C
Boiling point	77.3 °C	145 °C	240 °C	68.74 °C	188.53 °C	86.7 °C	137–144.5 °C
Density (20 °C)	0.806 g/cm ³	0.898 g/cm ³	0.99 g/cm ³	0.6603 g/cm ³	0.8264 g/cm ³	1.46 g/cm ³	0.861–0.880 g/cm ³
Vapour pressure	12 kPa (20 °C)	0.5 kPa (20 °C) 0.727 kPa (25 °C)	0.013 kPa (25 °C)	20 kPa (25 °C)	0.027 kPa (20 °C) 0.014 kPa (25 °C)	0.86 kPa (20 °C)	0.767 kPa–0.833 kPa (20 °C) 0.881 kPa–1.18 kPa (25 °C)
Log K _{oc} (average values)	1.06	88	n.a.	3.09–3.61	n.a.	2.1	2.11–2.31
Log K _{ow} (25 °C) (average values)	0.25	2.38	4.81	3.29	3.11	2.29	3.12–3.20
Solubility	Soluble in water (75.1 g/L (25 °C)) Miscible with many organic solvents such as alcohols, ethers, acetone, carbon tetrachloride, ethyl acetate, ethylene cyanohydrin, toluene, and aliphatic hydrocarbon solvents	Very slightly soluble in cold water (1.4 g/L (20 °C), 2 g/L (25 °C)) Soluble in ethanol, diethyl ether and acetone	Insoluble in water (5.33 × 10 ^{−3} g/L (25 °C)) Soluble in alcohol, acetone, benzene, carbon tetrachloride, castor oil, hexane and xylene	Insoluble in water (9.5 × 10 ^{−3} g/L (20 °C)) Very soluble in alcohol and miscible in chloroform, ether and other organic solvents	Limited solubility in water (0.572 g/L (25 °C))	Slightly soluble in water (1.1 g/L (25 °C)) Miscible with very common organic solvents (e.g. ether, alcohol, chloroform)	Insoluble in water (0.106–0.178 g/L) Miscible with alcohol, ether and other organic solvents
Uses	• As co-monomer in the production of acrylic and modacrylic fibres (used for clothing, carpeting and other fabrics and in the production of rugged plastics for automotive components, computers, appliance) • Production of plastics, surface coatings, nitrile elastomers, barrier resins, and adhesives • Synthesis of various antioxidants, pharmaceuticals, dyes and surface-active agents	• As intermediate in organic synthesis • Production of polymers and resins for textile and leather finishings, solvent coatings, adhesives, paint binders and emulsifiers	• Synthesis in the chemical industry and as solvent for various products	• In food processing, including extraction of vegetable oil from soybeans, flaxseed, peanuts, safflower seed, corn germ, and cottonseed • As polyolefins solvent, a cleaning agent, a rubber polymerization solvent, a laboratory chemical • Used in low-temperature thermometers and in the manufacture of pharmaceuticals	• Raw material for surface active agents and plasticizer, perfume (used in many fragrance compounds [e.g. body lotion, shampoo, eau de toilette]) and solvents	• Vapour degreasing and cleaning of metal parts • Synthesis in the chemical industry and as solvent for various products • Contained in some household products, including typewriter correction fluid, paint removers, adhesives, and spot removers	• As a solvent, a cleaning agent, and a paint thinner • In printing, rubber, and leather industries • To a lesser extent as material in chemical, plastics, and synthetic fiber industries and as an ingredient in the coating of fabrics and papers • Found in small amounts in airplane fuel and gasoline
Hazards	Flammability, polymerization activity and toxicity (environmental effects and human health effects)	Flammability, polymerization activity and toxicity (environmental effects and human health effects)	Flammability and toxicity (environmental effects and human health effects)	Flammability and toxicity (environmental effects and human health effects)	Flammability and toxicity (environmental effects and human health effects)	Toxicity (environmental effects and human health effects)	Flammability and toxicity (environmental effects and human health effects)
Persistence	Biologically degradable (t _{1/2} in water: 170 h (30–552)) – not expected to be persistent in the environment Hydrolysis very slow (t _{1/2} 13 and 188 years, in acidic and basic conditions, respectively)	Readily biodegradable Hydrolysis very slow (t _{1/2} 1100 days)	Not readily biodegradable	In water: loss mainly by volatilisation but also by adsorption to sediment or suspended particulate matter and biodegradation	Readily biodegradable in water – not expected to be hydrolyzed and biodegradable under aerobic conditions Volatilisation from water to air is not considered to be an important fate process	Moderate persistence In water: loss mainly by volatilisation, although it can also be biodegraded	In water: loss mainly by volatilisation
Bioaccumulation	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 48)	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 13)	Moderate potential to bioaccumulate or bioconcentrate in aquatic organisms	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 174–776)	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 3.9–8.1)	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 17–39)	Low potential to bioaccumulate or bioconcentrate in aquatic organisms (e.g. BCF = 6–23)
Property group (European Classification System for chemicals)	Floater, dissolver and evaporator	Floater, dissolver and evaporator	Floater	Floater and evaporator	Floater	Sinker and dissolver	Floater and evaporator

were found (Table 4). The extensive use of acrylonitrile, trichloroethylene and xylene in industry, their ubiquitous presence in aquatic systems due to industrial discharges (Lech et al., 1996; Schultz et al., 1996; Dobaradaran et al., 2012) and potential carcinogenicity (Manning et al., 2001; Neuparth et al., 2013) are possible explanations for such differences. Furthermore, in terms of ecotoxicological impact of the selected HNS, mortality has been the effect most frequently assessed (34%) followed by evaluations on biomass and growth (19%) (Fig. 1). Embryotoxicity studies (10%, Fig. 1) were found for all HNS with the exception of isononanol (Tables 2 and 3). These bioassays were performed with fertilised eggs and larvae of either invertebrates (McGowan et al., 2013) or vertebrates (Fort et al., 1993; Tong et al., 1996a; Hayashi et al., 1998; McDaniel et al., 2004; McGowan et al., 2013). Fewer studies were however carried out to evaluate potential alterations in reproduction (7%) and carcinogenicity (3%) of freshwater and seawater organisms, as well as, in biochemical, histochemical, histological and behavioural biomarkers. The latter accounted for 16% of the reviewed literature only when grouped in the same category showing once more the dearth of knowledge and the need to conduct further studies.

Compiled data suggest that HNS ecotoxicity is dependent on several features such as HNS nature, the tested species and experimental conditions. The developmental stage is a pivotal feature in these type of studies as sensitivity of a given species is known to vary according to different stages through its life cycle (ITOPF, 2011). Exposure conditions are another important issue. Effectively, in experiments with rotifers carried out in similar conditions but by different laboratories, the results were consistent for both freshwater (*B. calyciflorus*) and seawater (*B. plicatilis*) species. In exposures of *B. calyciflorus* to hexane, the 24 h-LC₅₀ was 68 (40–89) mg/L according to Snell et al. (1991a) and 68.3 (57.9–78.7) mg/L according to Ferrando and Andreu-Moliner (1992). In exposures of *B. plicatilis* to the same HNS, the 24 h-LC₅₀ was 154 (126–182) mg/L according to Snell et al. (1991b) and 154 (145–160) mg/L according to Ferrando and Andreu-Moliner (1992) (Table 2). This also highlights the importance of systematising the experimental conditions and performing ecotoxicological bioassays with marine species for accurate exposure assessment and risk calculation. Standard test guidelines (e.g. standardised methods of OECD, U.S. EPA and American Water Works Association) were used in some cases with water flea (*Daphnia magna* (Tong et al., 1996b; Di Marzio and Saenz, 2006; Dobaradaran et al., 2012)) and *Bryconamericus iheringii* (Di Marzio and Saenz, 2004). Nevertheless, for some of the data compiled in Tables 2 and 3 experimental conditions were not always described in detail. This lack of information makes it difficult to understand certain differences, such as those found in acute toxicity of acrylonitrile (48 h-LC₅₀: 22.0 mg/L and 8.7 mg/L) (Tong et al., 1996a; EURAR, 2004a) and n-butyl acrylate (48 h-LC₅₀: 8.2 mg/L, 19.8 mg/L and 5.2 mg/L) (OECD-SIDS, 2002a; MOE, 2014a) towards water flea (Table 2).

Previous studies indicated differential sensitivity among species, particularly when comparing freshwater to marine data (Tables 2 and 3) to the HNS in question. For instance, *Daphnia* tests following standardised procedures (Tong et al., 1996b; Di Marzio and Saenz, 2006; Dobaradaran et al., 2012) showed different sensitivity to acrylonitrile (Tong et al., 1996a, b; EURAR, 2004a), n-butyl acrylate (OECD-SIDS, 2002a), isononanol (OECD-SIDS, 2002b), trichloroethylene (Dobaradaran et al., 2012) and xylene (Zhao et al., 1995) (Table 2). In addition, in experiments assessing the effect of n-butyl acrylate,

cyclohexylbenzene, hexane and trichloroethylene on the length of the longest arm of sea urchin larvae and on embryo malformations in turbot (*Scophthalmus maximus*) eggs, McGowan et al. (2013) recorded considerable differences (in some cases an order of magnitude) in NOEC and LOEC values found for each HNS. Sub-lethal effects (e.g. biochemical, physiological, reproductive) may also be triggered by longer exposures. For example, exposure of seabass (*Dicentrarchus labrax*) to acrylonitrile for 15 days elicited induction of antioxidant enzymes, while exposure of marine amphipods to p-xylene for 36 days, induced the activity of antioxidant enzymes, altered the growth rate and biased the sex-ratio (Neuparth et al., 2013, 2014). Such alterations further reinforce the need to assess effects towards different marine species.

Contaminants are known to induce stress and, consequently, changes in the exposed organisms that can reduce their overall condition and ultimately their ability to reproduce, grow, feed and/or function normally (ITOPF, 2011). Different biomarkers have been employed to assess the toxicity of pollutants in aquatic organisms (e.g. antioxidant enzymes, cytochrome P450 isoenzymes) (Garrigues et al., 2001; Bairy and Marques, 2003) and are recommended for use within the scope of the Marine Strategy Framework Directive in integrated monitoring programmes aiming at assessing the *Good Environmental Status* (GES) (Davies and Vethaak, 2012). Biomarkers can serve as diagnostic and prognostic early warning signals for exposure to pollutants before adverse effects on individual animals or populations are observed (Abrahamson, 2007).

With regard to HNS, biomarkers are still poorly used as indicators of exposure and effects induced in the organisms: only a limited number of studies were in fact found (Tables 2 and 3). These few studies addressed changes in organisms' behaviour elicited by HNS. Behaviour links physiological function with ecological processes, constituting an adequate indicator of toxicity of pollutants (Scott and Sloman, 2004). For instance, alterations in the ability to maintain balance have been reported for water flea exposed to trichloroethylene (ECOTOX, 2014). For vertebrates, rainbow trout (*Oncorhynchus mykiss*) showed signs of surfacing, laboured respiration, quiescence, on-bottom orientation and loss of equilibrium when exposed to n-butyl acrylate (OECD-SIDS, 2002a). Tilapia (*Tilapia zillii*) and Japanese medaka (*Oryzias latipes*) showed changes in their feeding behaviour after 96 h or 15 days of exposure to xylene and isononanol, respectively (OECD-SIDS, 2002b; MOE, 2009). Increased erratic movement and ventilator frequency and changes in swimming patterns were observed in bluegill (*Lepomis macrochirus* (Diamond et al., 1990)) and rainbow trout (Kaiser et al., 1995) after a couple of hours of exposure to trichloroethylene. With only a few studies available, the information is still very limited relative to histological and histochemical changes induced by HNS (Scott et al., 2002; Agwuocha et al., 2011). Similarly, such paucity of knowledge was also encountered for carcinogenic effects. Only studies evaluating acrylonitrile carcinogenicity in fish could be found. This HNS was shown to induce proliferation of hepatocytes in Japanese medaka (Ortego et al., 1996) and guppy (*Poecilia reticulata*) (Hawkins, 1991). No correlation was however found to carcinogenesis so the authors considered that HNS cytotoxic but non-carcinogenic to both fish species. Nevertheless, evidence of HNS carcinogenicity can be found in literature as discussed below (IARC, 1995, 1999; EURAR, 2004a; Abele et al., 2011). Therefore, research must be pursued in an attempt to clarify the knowledge about carcinogenic effects of HNS on aquatic organisms, especially marine ones.

Notes to Table 1:

n.a. – not available; BCF – bioaccumulation factor.

^a e ANGroup (2012), EURAR (2004a); Long et al. (2002).

^b OECD-SIDS (2002a).

^c ACROS (2009), ChemIDplus (2014).

^d ATSDR (1999), CE (2004).

^e OECD-SIDS (2002b), McGinty et al. (2010).

^f IARC (1995); EURAR (2004b).

^g ATSDR (2007), van Leeuwen (2009).

Table 2
 Toxicological endpoints reported in literature for aquatic algae and plants and invertebrates exposed, acutely or chronically, to hazardous and noxious substances (HNSs), such as, acrylonitrile, butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene.

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
Acrylonitrile Algae/plant	Duckweed (FW) <i>Lemna minor</i>	Exposure type: SS 96 h	Growth inhibition NOEC, LOEC	96 h-NOEC: 6.25 96 h-LOEC: 12.5	Tong et al. (1996a)
		Exposure type: SS 96 h [P] 0–50 mg/L	Growth inhibition IC ₅₀ , NOEC, LOEC	96 h-IC ₅₀ : 27.08 96 h-NOEC: 6.2 96 h-LOEC: 12.5	Tong and Hongjun (1997)
	Green alga (FW) <i>Scenedesmus subspicatus</i>	72 h	Growth Biomass EC ₅₀ , NOEC, LOEC	Biomass 72 h-EC ₅₀ : 3.1 Growth 72 h-EC ₅₀ : >7.1 Growth 72 h-NOEC: 0.8 Growth 72 h-LOEC: 1	Bayer (1995)
	Diatom (SW) <i>Skeletonema costatum</i>	72 h	Growth Biomass EC ₅₀ , NOEC	Growth 72 h-EC ₅₀ : 14.1 Growth 72 h-NOEC: 3.0 Biomass 72 h-EC ₅₀ : 1.63 Biomass 72 h-NOEC: 0.41	ANGroup (1997)
Annelida	Tubificid worm, Oligochaete (FW) <i>Limnodrilus hoffmeisteri</i>	Organisms 1–2 cm Exposure type: S 96 h	Immobilization EC ₅₀	96 h-EC ₅₀ : 16.90 (15.87–17.88)	Tong et al. (1996a)
Mollusca	Mollusc (FW) <i>Radix plicatula</i>	Juvenile (3–4 days) Exposure type: S 48 h, 96 h	Immobilization EC ₅₀	48 h-EC ₅₀ : 39.97 96 h-EC ₅₀ : 17.94	Tong et al. (1996a)
Arthropoda – Insecta	Midge (FW) <i>Chironomus sp.</i>	Larvae (<24 h) Exposure type: S 48 h	Immobilization EC ₅₀	96 h-EC ₅₀ : 14.21 (9.45–21.36)	Tong et al. (1996a)
Arthropoda – Crustacean	Brine shrimp (SW) <i>Artemia salina</i>	Neonates (<24 h) Exposure type: SS 48 h	Immobilization EC ₅₀	48 h-EC ₅₀ : 13.34 (12.58–14.12)	Tong et al. (1996a)
		Water flea (FW) <i>Daphnia magna</i>	48 h	Mortality LC ₅₀	48 h-LC ₅₀ : 22.0
		Neonates (<24 h) Exposure type: SS 48 h, 21 days	Immobilization, EC ₅₀ Reproduction Mortality NOEC, LOEC	48 h-EC ₅₀ : 8.697 (7.38–10.25) Reproduction 21 d-NOEC: 0.5 Reproduction 21 d-LOEC: 1.0 Mortality 21 d-NOEC: 2.0 Mortality 21 d-LOEC: n.a.	Tong et al. (1996a)
	Organisms <24 h Exposure type: SS 48 h, 14 and 21 days	Immobilization, EC ₅₀ Reproduction Mortality NOEC, LOEC	48 h-EC ₅₀ : 10 Reproduction 21 d-NOEC: 0.5 Reproduction 21 d-LOEC: 1.0 Mortality 21 d-NOEC: 2.0 Mortality 21 d-LOEC: >4.0	Tong et al. (1996b)	
N-butyl acrylate Algae/plant	Green alga (FW) <i>Desmodesmus subspicatus</i>	72 h [Iso-butyl acrylate] 0–100 mg/L	Growth Biomass EC ₅₀ , NOEC, LOEC	Biomass 72 h-EC ₅₀ : 3.18 (mc) Growth 72 h-EC ₅₀ : 5.28 (mc) Growth 72 h-NOEC: 0.82 Growth 72 h-LOEC: 1.65	BASF (2002)
		Macroalga (SW) <i>Fucus vesiculosus</i> Green alga (FW) <i>Selenastrum capricornutum</i>	Exposure type: SS 96 h Exposure type: S 96 h [N-butyl acrylate] 3.8–60 mg/L	FronD growth and successful germination NOEC Growth EC ₅₀ , NOEC, LOEC	Growth 96 h-NOEC <60 Germination 96 h-NOEC <60 96 h-EC ₅₀ : 5.2 (nc) 96 h-NOEC <3.8 96 h-LOEC <3.8
	Annelida	Marine bristle worm (SW) <i>Pomatoceros triquetter</i>	Fertilized eggs 48 h	Number of normally developed and abnormal or underdeveloped embryos EC ₅₀ , NOEC, LOEC	48 h-EC ₅₀ : 10.6 (7.49–13.89) 48 h-NOEC <10 48 h-LOEC: 10
Arthropoda – Crustacean	Water flea (FW) <i>Daphnia magna</i>	Exposure type: F 4–48 h [N-butyl acrylate] 1.2–20 mg/L	Immobilization EC ₅₀ , NOEC	4 h-EC ₅₀ >17 24 h-EC ₅₀ >17 48 h-EC ₅₀ : 8.2 (7.3–9.3) (mc) 48 h-NOEC: 2.4	Burgess (1990)
		Organisms of 2–24 h 3–48 h	Immobilization EC ₅₀	3 h-EC ₅₀ >100 6 h-EC ₅₀ >100	BASF (1991)

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Table 2 (continued)

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
		[Iso-butyl acrylate] 3.13–100 mg/L		24 h-EC ₅₀ : 36.7 (32.7–41.1) (nc) 48 h-EC ₅₀ : 19.8 (16.4–24.0) (nc) 48 h-EC ₅₀ : 5.2 21 d-EC ₅₀ > 1.0 21 d-NOEC: 1.0	MOE (2014a)
	Copepod (SW) <i>Tisbe battagliai</i>	Juveniles Exposure type: SS 48 h 3 repetitions	Immobilization EC ₅₀ Reproduction EC ₅₀ , LOEC Mortality LC ₅₀ , NOEC, LOEC	48 h-LC ₅₀ (1): 15.7 (12.3–20.1) 48 h-NOEC (1): 6 48 h-LOEC (1): 10 48 h-LC ₅₀ (2): 2.53 (1.19–4.00) 48 h-NOEC (2) < 3 48 h-LOEC (2): 3 48 h-LC ₅₀ (3): 4.2 (1.01–3.77) 48 h-NOEC (3): 3 48 h-LOEC (3): 10 48 h-NOEC: 0.158 48 h-LOEC: 0.237	McGowan et al. (2013)
Echinoidea	Sea urchin (SW) <i>Paracentrotus lividus</i>	Larvae Exposure type: S 48 h [N-butyl acrylate] 0.105–0.800 mg/L	Length of the longest arm of each larva NOEC, LOEC	48 h-NOEC: 0.158 48 h-LOEC: 0.237	McGowan et al. (2013)
Cyclohexylbenzene Arthropoda – Crustacean	Water flea (FW) <i>Daphnia pulex</i>	Neonates (<24 h) Exposure type: S 48 h 48 h	Immobilization EC ₅₀	48 h-EC ₅₀ : 0.55 (mc)	Passino-Reader et al. (1997)
Echinoidea	Sea urchin (SW) <i>Paracentrotus lividus</i>	Larvae Exposure type: S 48 h [P] 0.439–2.222 mg/L	Immobilization EC ₅₀ Length of the longest arm of each larvae NOEC, LOEC	48 h-EC ₅₀ : 0.37 48 h-NOEC: 0.658 48 h-LOEC: 0.988	MOE (2014b) McGowan et al. (2013)
Hexane Rotifera	<i>Brachionus calyciflorus</i> (FW)	Neonates Exposure type: S 24 h	Mortality LC ₅₀	24 h-LC ₅₀ : 68 (49–89) 24 h-LC ₅₀ : 68.3 (57.9–78.7)	Snell et al. (1991a) Ferrando and Andreu-Moliner (1992)
	<i>Brachionus plicatilis</i> (SW)	Neonates Exposure type: S 24 h	Mortality LC ₅₀	24 h-LC ₅₀ : 154 (126–182) 24 h-LC ₅₀ : 154 (145.5–160)	Snell et al. (1991b) Ferrando and Andreu-Moliner (1992)
Echinoidea	Sea urchin (SW) <i>Paracentrotus lividus</i>	Larvae Exposure type: S 48 h [P] 9.2–70.0 mg/L	Length of the longest arm of each larvae NOEC, LOEC	48 h-NOEC < 70.0 48 h-LOEC < 70.0	McGowan et al. (2013)
Isononanol Algae/plant	Green alga (FW) <i>Raphidocelis subcapitata</i>	Exposure type: S 72 h [P] 2.13–50.0 mg/L	Growth Biomass EC ₅₀ , NOEC	Growth 72 h-EC ₅₀ > 50 (nc) Growth 72 h-NOEC: 10.3 (nc) Biomass 72 h-EC ₅₀ : 19.5 (14.8–25.8) (nc) Biomass 72 h-NOEC: 4.7 (nc)	OECD-SIDS (2002b)
Arthropoda – Crustacean	Water flea (FW) <i>Daphnia magna</i>	<24 h after hatching Exposure type: SS 24 h, 48 h [P] 4.94–25.0 mg/L <24 h after hatching Exposure type: F 21 days [P] 0.128–5.0 mg/L	Immobilization EC ₅₀ , LOECi (100% immobilization) Mortality Reproduction LC ₅₀ , EC ₅₀ , NOEC, LOEC	24 h-EC ₅₀ : 9.24 (8.08–10.6) 48 h-EC ₅₀ : 6.77 (5.88–7.71) 48 h-LOECi: 21.8 21 d-LC ₅₀ > 3.87 21 d-EC ₅₀ : 2.09 (1.94–2.25) 21 d-NOEC: 1.46 21 d-LOEC: 3.87	OECD-SIDS (2002b) OECD-SIDS (2002b)
Trichloroethylene Algae/plant	Green alga (FW) <i>Chlamydomonas reinhardtii</i>	Exposure type: S 72 h Exposure type: S 2 h Green alga (FW)	Biomass EC ₅₀ Biochemical effects (chlorophyll fluorescence) EC5 Histological effects (proliferation of cells and	72 h-EC ₅₀ : 36.5 2 h-EC5: 13 24 h-EC10: 70 and 82	Brack and Rottler (1994) Brack and Frank (1998) ECOTOX (2014a) and references

	<i>Scenedesmus subspicatus</i>	24 h and 96 h	morbid cysts) EC10	96 h-EC10: 46 and 61	therein
	Green algae (FW) <i>Chlorella vulgaris</i> <i>Scenedesmus quadricauda</i> <i>Raphidocelis subcapitata</i>	Exposure type: S 120 h [P] 0.005–0.500 mg/L	Amount of test chemical remaining in tissue after exposure NOEC	120 h-NOEC < 0.005	Smets and Rittmann (1990)
	Green alga (FW) <i>Chlamydomonas reinhardtii</i> , <i>Chlorella kessleri</i> , <i>Desmodesmus subspicatus</i> , <i>Desmodesmus quadricauda</i> , <i>Raphidocelis subcapitata</i>	72 h (tests carried out in immunological plates and glass enclosures)	Cell density, biomass, O ₂ production or pH increment EC ₅₀	Immunological plates Biomass 72 h-EC ₅₀ : 240–800 Glass enclosures Biomass 72 h-EC ₅₀ : 130–820 O ₂ production 27 h-EC ₅₀ : 100–700 pH 72 h-EC ₅₀ : 250–700	Lukavský et al. (2011)
	Cyanobacteria (FW) <i>Synechococcus elongatus f. thermalis</i> , <i>Synechococcus leopoliensi</i> , <i>Microcystis aeruginosa</i>				
	Algae (FW) <i>Chlorella ellipsoidea</i> , <i>Chlorococcum</i> sp., <i>Gleocystis ampla</i> , <i>Nannochloris</i> sp., <i>Scenedesmus obliquus</i> , <i>Tetraselmis</i> sp.	Exposure type: S 96 h [P] 0.05–0.2% (v/v)	Growth of cultures LOEC	96 h-LOEC > 0.05% (v/v) (<i>Gleocystis ampla</i> , <i>Scenedesmus obliquus</i> , <i>Chlorococcum</i> sp.)	Tadros et al. (1994)
	Diatom (SW) <i>Cyclotella</i> sp., <i>Cylindrotheca</i> sp., <i>Nitzschia dissipata</i> , <i>Nitzschia pusilla</i> sp., <i>Thalassiosira weissflogii</i>	Exposure type: S 96 h [P] 0.2–0.3% (v/v)	Growth of cultures LOEC	96 h-LOEC > 0.3% (v/v) Except for: <i>Nitzschia dissipata</i> ; <i>Thalassiosira weissflogii</i> , <i>Nitzschia pusilla</i> sp. 96 h-LOEC > 0.2% (v/v)	Tadros et al. (1995)
Trichloroethylene	Phytoplankton (FW)	Exposure type: E 11 weeks [P] 1.5 and 7.5 mg/L	Algae abundance, ATP, primary, productivity NOEC	11 w-NOEC < 1.5	Lay and Herrmann (1991)
Rotifera	Rotifer (FW)	Exposure type: E 11 weeks [P] 1.5 and 7.5 mg/L	Biomass NOEC	11 w-NOEC < 1.5	Lay and Herrmann (1991)
Mollusca	Clam (FW) <i>Corbicula fluminea</i>	Adults Exposure type: S 120 h [P] 1.56–100 mg/L	Components of (de)toxication metabolism of phases I and II, parameters related to oxidative stress and propionylcholinesterase activity NOEC or LOEC	CAT 120 h-NOEC < 1.2 PL 120 h-NOEC < 1.2 P450 120 h-LOEC ≥ 3.6 NADH 120 h-LOEC ≥ 14	Vidal et al. (2001)
Arthropoda – Crustacean	Cyclopoid copepod (FW) <i>Cyclops</i> sp.	Exposure type: E 11 weeks [P] 1.5 and 7.5 mg/L	Biomass NOEC	11 w-NOEC < 1.5	Lay and Herrmann (1991)
	Water flea (FW) <i>Daphnia magna</i>	Exposure type: S 24 h Neonates (<24 h) 2, 4, 6, 8, 24, 48, 72 and 96 h	Behavioural effects (change in the ability to maintain balance) EC ₅₀ Mortality LC ₅₀	33 24 h-LC ₅₀ : 43.14 (33.17–56.08) 48 h-LC ₅₀ : 33.85 (26.05–42.93) 72 h-LC ₅₀ : 28.39 (21.74–35.47) 96 h-LC ₅₀ : 26.55 (20.22–32.98) 72 h-NOEC: 1.384	OECD-SIDS (2002b) and references therein Dobaradaran et al. (2012)
		Exposure type: R 21 days	Behavioural effects (change in the ability to maintain balance) Reproduction NOEC		OECD-SIDS (2002b) and references therein
		Exposure type: R 21 days	Reproduction NOEC, LOEC	21 d-NOEC: 2.3 21 d-LOEC: 8	OECD-SIDS (2002b) and references therein
	Water flea (FW) <i>Daphnia pulex</i>	Exposure type: E 11 weeks [P] 1.5 and 7.5 mg/L	Biomass NOEC	11 w-NOEC < 1.5	Lay and Herrmann (1991)
Echinoidea	Sea urchin (SW) <i>Paracentrotus lividus</i>	Larvae Exposure type: S 48 h [P] 0.79–6.0 mg/L	Length of the longest arm of each larvae NOEC, LOEC	48 h-NOEC: 1.2 48 h-LOEC: 2.8	McGowan et al. (2013)
Xylene					
Bacteria	<i>Photobacterium phosphoreum</i> (SW)	5–15 min 15–30 min	Microtox test utilising the bioluminescent LC ₅₀ Inhibition of bioluminescence	LC ₅₀ : 8.5 15 min-EC ₅₀ : 22–23	Calleja et al. (1994) Zhao et al. (1995)

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Table 2 (continued)

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
Ciliophora	Ciliate protozoa (FW) <i>Tetrahymena pyriformis</i>	<i>m</i> -, <i>p</i> -, <i>o</i> -Xylene 48 h	EC ₅₀ Growth	48 h-EC ₅₀ : 88.1 (81.67–99.52)	Schultz et al. (1996)
Algae/plant	Green alga (FW) <i>Chlamydomonas reinhardtii</i>	Exposure type: S 2 h	Biochemical effects (chlorophyll fluorescence) EC20	2 h-EC20: 12	(Brack and Frank, 1998)
	Green alga (FW) <i>Raphidocelis subcapitata</i>	<i>m</i> -Xylene Exposure type: S 8 days	Growth EC ₅₀	<i>m</i> -Xylene 8 d-EC ₅₀ : 3.9 <i>p</i> -Xylene 8 d-EC ₅₀ : 4.4 <i>o</i> -Xylene 8 d-EC ₅₀ : 4.2	(Brack and Frank, 1998)
	Green alga (FW) <i>Scenedesmus quadricauda</i>	Exposure type: S 24 h	Growth EC ₅₀	<i>m</i> -Xylene 24 h-EC ₅₀ : 7.4 <i>p</i> -Xylene 24 h-EC ₅₀ : 9.5 <i>o</i> -Xylene 24 h-EC ₅₀ : 27.6	Di Marzio and Saenz (2006)
Rotifera	<i>Brachionus calyciflorus</i> (FW)	Exposure type: S 24 h 48 h	Mortality LC ₅₀ Reproduction EC ₅₀ , NOEC, LOEC	24 h-LC ₅₀ : 253 48 h-NOEC: 20 48 h-LOEC: 40 48 h-LC ₅₀ : 99	Burbank and Snell (1994) and references therein Snell and Moffat (1992)
		Cysts Exposure type: S 24 h	Mortality LC ₅₀	24 h-LC ₅₀ : 257.7 (203.9–301.5)	Ferrando and Andreu-Moliner (1992)
		Exposure type: S 0.5 h	Biochemical effects (enzymatic activity of esterase and enzyme phospholipase A2 (PLA2)) NOEC	Esterase-NOEC: 120 PLA2-NOEC: 190	Burbank and Snell (1994)
		<i>p</i> -Xylene Exposure type: S 24 h, 48 h	Ingestion rate Reproduction rate LC ₅₀ , NOEC	Ingestion rate-NOEC: 30 Reproduction-NOEC: 20	Juchelka and Snell (1994)
		24 h Rotokit F test	Mortality LC ₅₀	24 h-LC ₅₀ : 310	Calleja et al. (1994)
	<i>Brachionus plicatilis</i> (SW)	Neonates (0–2 h) Exposure type: S 24 h	Mortality LC ₅₀	LC ₅₀ : 496 (387–605)	Snell et al. (1991b)
		Cysts Exposure type: S 24 h	Mortality LC ₅₀	24 h-LC ₅₀ : 495.9 (461.8–530.1)	Ferrando and Andreu-Moliner (1992)
Xylene Mollusca	Snail (FW) <i>Amphimelania holandri</i> Fér.	Exposure type: SS 24, 48, 72, 96 and 120 h [xylene] 0.03–0.3% (v/v)	Mortality LC ₅₀	24 h-LC ₅₀ : 0.2070% (v/v) 48 h-LC ₅₀ : 0.0850% (v/v) 72 h-LC ₅₀ : 0.0500% (v/v) 96 h-LC ₅₀ : 0.0350% (v/v) 120 h-LC ₅₀ : 0.0260% (v/v)	Erben and Pišl (1993)
	Snail (FW) <i>Lymnaea stagnalis</i> L.	Exposure type: SS 24, 48, 72, 96 and 120 h [xylene] 0.03–0.3% (v/v)	Mortality LC ₅₀	24 h-LC ₅₀ : 0.1720% (v/v) 48 h-LC ₅₀ : 0.0930% (v/v) 72 h-LC ₅₀ : 0.0650% (v/v) 96 h-LC ₅₀ : 0.0500% (v/v) 120 h-LC ₅₀ : 0.0410% (v/v)	Erben and Pišl (1993)
	Clam (SW) <i>Gafrarium divaricatum</i>	Active clams, with protruding siphon and foot, of more or less uniform size (30–32 mm) Exposure type: SS 30 days [xylene] 4.25–8.5 mg/L	Histopathological changes in tissue (loss of bubbling epithelium, reduction in cytoplasm volume and density, fusion of cell membranes and nuclei forming darkly stained area at basal part of the cells) NOEC	30d-NOEC <4.25	Agwuocha et al. (2011)
Arthropoda – Crustacean	Brine shrimp (SW) <i>Artemia franciscana</i>	Nauplii Exposure type: S 24 h [<i>p</i> -Xylene] 15–35 mg/L Nauplii Exposure type: SS 31 days	Mortality LC ₅₀ Ecological parameters: survival, sex ratio, individual growth and reproductive traits (percentage of gravid females, length of gravid	24 h-LC ₅₀ : 17.7 (16.9–18.5) Survival 31 d-LOEC: 0.032 Growth 31 d-NOEC: 0.8 Growth 31 d-LOEC: 4	Neuparth et al. (2011a) Neuparth et al. (2011a)

	[p-Xylene] 0.032–4.0 mg/L	females and fecundity) Biomarkers: oxidative stress enzymes activity (catalase (CAT), glutathione-s-transferase (GST)) LOEC, NOEC	Reproduction 31 d-NOEC: 0.032 Reproduction 31 d-LOEC: 0.16 CAT 31 d-LOEC: 0.8 (males) GST 31 d-LOEC: 0.16 (females) 24 h-LC ₅₀ : 88	Calleja et al. (1994)
Artemia (SW) <i>Artemia salina</i>	24 h Artoxkit M test	Mortality LC ₅₀		
Water flea (FW) <i>Ceriodaphnia dubia</i>	Exposure type: S 48 h m-Xylene	Immobilization LC ₅₀	48 h-IC ₅₀ : 2.4 (1.6–3.2)	Rose et al. (1998)
Water flea (FW) <i>Daphnia magna</i>	Exposure type: S 24 h m-, p-, o-Xylene	Immobilization LC ₅₀	24 h-IC ₅₀ : 9–19	Zhao et al. (1995)
	24 h Microtox	Mortality LC ₅₀	24 h-LC ₅₀ : 75	Calleja et al. (1994)
Water flea (FW) <i>Daphnia spinulata</i>	Neonates (<24 h) Exposure type: S 48 h m-, p-, o-Xylene	Immobilization LC ₅₀	m-Xylene 48 h-IC ₅₀ : 4.2 p-Xylene 48 h-IC ₅₀ : 4.2 o-Xylene 48 h-IC ₅₀ : 6.4	Di Marzio and Saenz (2006)
Isopod (FW) <i>Asellus aquaticus</i> L.	Exposure type: SS 24, 48, 72, 96 and 120 h [Xylene] 0.01–0.07% (v/v)	Mortality LC ₅₀	24 h-LC ₅₀ : 0.0270% (v/v) 48 h-LC ₅₀ : 0.0230% (v/v) 72 h-LC ₅₀ : 0.0210% (v/v) 96 h-LC ₅₀ : 0.0200% (v/v) 120 h-LC ₅₀ : 0.0190% (v/v)	Erben and Pišl (1993)
Amphipod (FW) <i>Gammarus fossarum</i>	Exposure type: SS 24, 48, 72, 96 and 120 h [Xylene] 0.005–0.02% (v/v)	Mortality LC ₅₀	24 h-LC ₅₀ : 0.0184% (v/v) 48 h-LC ₅₀ : 0.0108% (v/v) 72 h-LC ₅₀ : 0.0079% (v/v) 96 h-LC ₅₀ : 0.0063% (v/v) 120 h-LC ₅₀ : 0.0053% (v/v)	Erben and Pišl (1993)
Amphipod (SW) <i>Gammarus locusta</i>	Juveniles (2–4 mm length class) Exposure type: SS 96 h [p-Xylene] 0.1–1.5 mg/L	Mortality LC ₅₀	96 h-LC ₅₀ : 1.1 (0.8–1.4)	Neuparth et al. (2011a)
Amphipod (FW) <i>Hyalella curvispina</i>	Organisms with 10 days Exposure type: F 96 h m-, p-, o-Xylene	Mortality LC ₅₀	m-Xylene 96 h-LC ₅₀ : 4.2 p-Xylene 96 h-LC ₅₀ : 4.2 o-Xylene 96 h-LC ₅₀ : 6.4	Di Marzio and Saenz (2006)
Fairy shrimp (FW) <i>Streptocephalus proboscideus</i>	24 h Streptoxkit F test	Mortality LC ₅₀	Artoxkit M 24 h-LC ₅₀ : 194	Calleja et al. (1994)

Nominal concentration (nc), measured concentration (mc). Hour (h), days (d), week (w). n.a. – not available.

^a Freshwater (FW); seawater (SW).

^b Static (S), semi-static (SS), flow-through (F), ex-situ bioassay (E), [P] – Concentration of the pollutant.

^c Effective concentration for x% of tested organisms (EC_x), lethal concentration for 50% of tested organisms (LC₅₀), inhibition concentration for 50% of tested organisms (IC₅₀), no observable effect concentration (NOEC), lowest observable effect concentration (LOEC).

^d 95% confident limits of LC₅₀, EC₅₀, IC₅₀, NOEC and LOEC were inserted between brackets whenever possible.

Furthermore, the uptake of contaminants by organisms instigates a series of internal mechanisms with the intent to reduce their cytotoxicity and cellular damage. Detoxification mechanisms (e.g. phase I and II biotransformation enzymes) are activated in specific sites of the cell to biotransform contaminants into more water-soluble metabolites. Antioxidant enzymes (e.g. catalase (CAT), superoxide dismutase (SOD)) and cofactors (e.g. reduced glutathione (GSH), vitamin E) are induced to reduce the oxidative stress that may be caused by the production of reactive oxygen substances (ROS) during biotransformation processes (Abele et al., 2011). Without such defence mechanisms, cell integrity would be disrupted, a cascade of events would follow the loss of cell vitality and the health of the organisms would be menaced. In this sense, the activity of enzymes involved in such processes and those associated with energetic metabolism (e.g. biotransformation enzymes, antioxidant enzymes, enzymes involved in aerobic/anaerobic energy metabolism and neurotransmission), the concentration of pivotal substances involved in important antioxidant reactions (e.g. GSH, cysteine) and evidence of cellular damage (e.g. lipid peroxidation, oxidation of proteins) have been used as biomarkers (Garrigues et al., 2001).

Evidence that HNS can trigger biochemical changes in exposed organisms is available in literature. Vidal et al. (2001) emphasised the relevance of cytochrome P450, NADH-cytochrome c reductase, catalase, peroxidised and peroxidisable lipids and net peroxidation as indicators of trichloroethylene hazard in Asian clam (*Corbicula fluminea*). Esterase and phospholipase A2 (PL2) activities were changed in the rotifer *B. calyciflorus* exposed to xylene (Burbank and Snell, 1994). For vertebrates, studies indicated that acrylonitrile and trichloroethylene may also induce oxidative stress and changes in activity of biotransformation enzymes in seabass (Neuparth et al., 2013) and rainbow trout (ECOTOX, 2014), respectively. Thus, several of those HNSs show toxic potential towards aquatic organisms highlighting the urge to establish early-warning tools, such as biomarkers, to monitor their presence and effects.

5. Relevant biomarkers of HNS toxicity

After chemical exposure, toxicological responses are triggered in organisms in consequence of a cascade of events related to the uptake, distribution and metabolism of the contaminant. The interaction of a chemical with the target sites and the imbalance produced in the organism when its protective physiological and biochemical defences are overwhelmed can induce stress (Freidig, 2000). Many chemicals are known to react with cellular macromolecules and to affect cell function and integrity (CBCSS-NRC, 2003), events that can be behind the perceptible toxicological hazards observed in exposed organisms. The identification of mechanisms of action of chemicals is therefore critical for understanding the complex relationship between the contaminant and the effects triggered. Furthermore, it provides parameters of interest to assess as biomarkers of exposure and effect.

The body of evidence with regard to the mechanisms of action of acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene is considerably limited and mainly focused on mammals (e.g. mice and rats (Farooqui and Ahmed, 1983; Fishbein, 1985; Kaneko et al., 1997; ATSDR, 1999; OECD-SIDS, 2002a, b; Pu et al., 2009)), with only a few studies available with aquatic organisms (Haydon and Urban, 1983; Lech et al., 1996; Vidal et al., 2001; Neuparth et al., 2013). Despite this, the available information greatly supports the identification of potential biomarkers of exposure to and effects of HNS, which could further be applied in the ERA of HNS.

5.1. Biotransformation and antioxidant defences

Once inside the organism, a contaminant will be distributed, accumulated and metabolised. Distribution of a chemical is conditioned by several factors including the route of exposure and the physico-chemical characteristics of the contaminant. For example, chemicals

capable of readily diffusing across membranes or binding to water-soluble compounds in blood or lymph can be more widely distributed in an organism (CBCSS-NRC, 2003). As mentioned above, the potential of the HNS investigated herein to bioaccumulate is low (Table 1). Hence, after ingestion, these chemicals are distributed through the organism and metabolised mainly in the liver or gastrointestinal tract (Davidson and Beliles, 1991; Bolt and Lewalter, 1993; IARC, 1995; ATSDR, 1999; EFSA, 2012; PubChem, 2014). The metabolism of acrylonitrile, trichloroethylene, hexane and xylene is fairly described in the literature. Though, this knowledge derives mainly from studies involving mammals and exposure through inhalation in preclinical and clinical studies (ATSDR, 1999, 2007; EURAR, 2004a,b). For cyclohexylbenzene, isononanol and n-butyl acrylate, the available information is even scarcer with regard to both aquatic organisms and mammals.

Metabolic processes of terrestrial and aquatic species present similarities sharing a set of biotransformation enzymes that occur in both groups (Netherton, 2011). This supports the prediction of HNS metabolism in aquatic species from information on mammalian metabolic pathways. Nevertheless, further research clarifying the metabolism of these contaminants in aquatic species is important, since differences in the specific nature and activity of detoxification enzymes, and/or the presence of alternative detoxification pathways, can influence the toxicokinetics of HNS.

In resemblance to other contaminants, some HNS can be metabolised through phase I and II biotransformation mechanisms: via cytochrome P450 and/or by conjugation with GSH either or not enzymatically assisted by glutathione-S-transferase (GST). This is the case of acrylonitrile (Lech et al., 1996; Kirman et al., 2005), n-butyl acrylate (Tyler et al., 1993), hexane (ATSDR, 1999), isononanol (SCBT, 2014), trichloroethylene (Barton et al., 1994) and xylene (Fishbein, 1985). Since biotransformation enzymes are activated throughout HNS metabolism, changes in their activity are expected upon direct exposure. Reports of such alterations have in fact been found in literature: the activity of phase I (cytochrome P450-dependent mixed-function oxidase system) and phase II (GST) enzymes increased in some aquatic organisms and mammals exposed to acrylonitrile (EURAR, 2004a), cyclohexylbenzene (PubChem, 2014), hexane (EHC, 1991; USEPA, 2005), trichloroethylene (Vidal et al., 2001; DuTeaux et al., 2003) and xylene (Al-Ghamdi et al., 2003a). Nevertheless, the role of cytochrome P450 isoenzymes is still inconclusive. Evidence that not all cytochrome P450 isoenzymes might be involved in the detoxification of these HNSs was found for both mammals and fish exposed to acrylonitrile. Wang et al. (2002) observed that the only enzyme responsible for acrylonitrile metabolism in mice was CYP2E1. In seabass liver, the same HNS had no significant effect in ethoxyresorufin-O-deethylase (EROD) activity probably indicating that the isoenzyme CYP1A had no active role on acrylonitrile biotransformation (Neuparth et al., 2013). For hexane, similar results were found in bioassays performed with rats: CYP2E1 activity was induced in hexane-treated rats but EROD activity was not increased. It was inferred that CYP2A1/2 and CYP2B1/2 activities were not induced by hexane (USEPA, 2005). On the other hand, CYP2B1/2 activity was induced to some extent in rats exposed to hexane so more than one isoenzyme could be probably involved in the biotransformation of this HNS (USEPA (2005) and references within). The available knowledge on HNS metabolism concerns mainly mammals. However, considering that conserved metabolic pathways are known to be present in aquatic organisms (Neuparth et al., 2013), the same enzymes might be also activated in marine organisms. Further research is therefore necessary to better understand the involvement of cytochrome P450 enzymatic system in the metabolism of these HNSs and others included in priority lists in marine organisms.

During the biotransformation process, HNS will be converted to different and more soluble chemical forms (metabolites) that can be more easily excreted and eliminated through urine and bile (CBCSS-NRC, 2003). The metabolism of xenobiotic compounds intends to facilitate their excretion and decrease their biological reactivity, boosting their

Table 3
Toxicological endpoints reported in literature for aquatic vertebrates exposed, acutely or chronically, to hazardous and noxious substances (HNSs), such as, acrylonitrile, butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene.

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
<i>Acrylonitrile</i>					
Chordata – Amphibia	Toad (FW) <i>Bufo bufo</i> ssp. <i>gargarizans</i>	Tadpoles (2–3 days) Exposure type: F 48 h, 96 h	Mortality LC ₅₀	48 h-LC ₅₀ : 14.22 (13.41–15.07) 96 h-LC ₅₀ : 11.59 (11.26–11.94)	Tong et al. (1996a)
		Tadpoles (2–3 days) Exposure type: F 21 days	Morphological (Leg development) NOEC, LOEC Mortality, LC ₅₀	48 h-NOEC: 3.2 (hind leg) 0.4 (fore leg) Mortality 48 h-LC ₅₀ : 3.2 48 h-LOEC: NR (hind leg) 0.8 (fore leg)	Tong et al. (1996a)
Chordata – fish	Grass carp, white Amur (FW) <i>Ctenopharyngodon idella</i>	Juveniles (3140 ± 610 mg) Exposure type: SS 48 h, 96 h	Mortality LC ₅₀	48 h-LC ₅₀ : 9.22 (8.95–9.49) 96 h-LC ₅₀ : 5.16 (4.96–5.38)	Tong et al. (1996a)
		Common carp (SW) <i>Cyprinus carpio</i>	Juveniles (31.8 ± 3.4 mg) Media type: SW Exposure type: R and S 48 h, 96 h	Mortality LC ₅₀	48 h-LC ₅₀ : 42.33 96 h-LC ₅₀ : 19.64 (18.07–21.36)
	Sheepshead minnow (FW, SW) <i>Cyprinodon variegatus</i>	Embryo-larval (<36 h) Exposure type: SS 7 days	Mortality NOEC, LOEC	7 d-NOEC: 1.6 7 d-LOEC: 3.2	Tong (1999)
		Organisms with 0.55–0.75 g Media type: SW Exposure type: SS 24 h, 48 h, 72 h, 96 h	Mortality LC ₅₀	24 h-LC ₅₀ : 28.2 48 h-LC ₅₀ : 15.9 72 h-LC ₅₀ : 15.9 96 h-LC ₅₀ : 8.6	EURAR (2004a) and references therein
	European seabass (SW) <i>Dicentrarchus labrax</i>	Juveniles (30–34 g) Exposure type: SS 96 h	Mortality LC ₅₀ , NOEC, LOEC	96 h-LC ₅₀ : 8.1 96 h-NOEC: 2.5 96 h-LOEC: 8.0	Neuparth et al. (2013)
		Juveniles (30–34 g) Exposure type: SS 22 days (15 d exposure; 7 d recovery) [P] 0–2 mg/L	Mortality Biochemical effects (activity of CAT, GST, superoxide dismutase (SOD) and Ethoxyresorufin-O-deethylase (EROD)) NOEC, LOEC	Mortality 22 d-NOEC: 0.15 Mortality 22 d-LOEC: 0.75 CAT 22 d-NOEC: 0.75 CAT 22 d-LOEC: 2 GST 22 d-NOEC: 0.15 GST 22 d-LOEC: 0.75	Neuparth et al. (2013)
	Rainbow trout (FW) <i>Oncorhynchus mykiss</i>	Organisms of 150 g Exposure type: S 24 h exposure [P] 1.002 µC ₁ /L	Biochemical effects (reaction of acrylonitrile with parvalbumin protein)	2 h-NOEC < 1.002 µC ₁ /L	Lech et al. (1996)
Japanese medaka (FW) <i>Oryzias latipes</i>	Organisms with 6 d Exposure type: multiple pulse tests 1, 2 or 4 times 24 h-exposure during 24 weeks (sampling 4, 6 and 12 months after exposure) [P] 35 mg/L 6–7 days post-hatch Exposure type: F 28 d [P] 5 mg/L	Induction of hepatic neoplasms NOEC	NOEC < 35 (not carcinogenic to medaka)	Hawkins (1991)	
Guppy (FW) <i>Poecilia reticulata</i>	Exposure type: multiple pulse tests 1, 2 or 4 times 24 h-exposure during 24 weeks (sampling 4, 6 and 12 months after exposure)	Induction of proliferation in hepatocytes (assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry) NOEC	28 d-NOEC < 5 (not carcinogenic to medaka)	Ortego et al. (1996)	
		Induction of hepatic neoplasms NOEC	NOEC < 35 (not carcinogenic to guppy)	Hawkins (1991)	

(continued on next page)

Table 3 (continued)

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
		[P] 35 mg/L			
<i>N-butyl acrylate</i> Chordata – fish	Sheepshead minnow (SW) <i>Cyprinodon variegatus</i>	Juveniles Exposure type: F 24, 48, 72 and 96 h [N-butyl acrylate] 0.011–0.09 mg/L	Mortality LC ₅₀ , NOEC	24 h-LC ₅₀ > 5.1 48 h-LC ₅₀ : 4.2 72 h-LC ₅₀ : 2.3 96 h-LC ₅₀ : 2.1 (1.3–3.5) 96 h-NOEC < 1.3	OECD-SIDS (2002a) and references therein
	Fathead minnow (FW) <i>Pimephales promelas</i>	Exposure type: S 96 h Iso-butyl acrylate	Mortality LC ₅₀	96 h-LC ₅₀ : 10–20	OECD-SIDS (2002b) and references therein
	Rainbow trout (FW) <i>Oncorhynchus mykiss</i>	Exposure type: F 24, 48, 72 and 96 h [N-butyl acrylate] 0.438–14 mg/L	Mortality, LC ₅₀ Behavioural/sub-lethal effects, NOEC Surfacing, laboured respiration, quiescence, on-bottom orientation and loss of equilibrium	24 h-LC ₅₀ > 7.2 48 h-LC ₅₀ > 7.2 72 h-LC ₅₀ : 6.6 96 h-LC ₅₀ : 5.2	OECD-SIDS (2002a) and references therein
	Turbot (SW) <i>Scophthalmus maximus</i>	Eggs Exposure type: 9 day exposure period, in four sampling points: 53 h postfertilization (hpf) (day 2) – 75% epiboly; 100 hpf (day 4) – before hatching (108 hpf)-after heart start beating (92 hpf); 124 hpf (day 5) – after hatching (108 hpf); and 220 hpf (day 9) – after mouth opening (204 hpf) [N-butyl acrylate] 0.031–1.2 mg/L	Embryo malformations – developmental delay, abnormal cellular masses, yolk sac alterations, oil globule fragmentation, oil globule position, no rupture of egg membrane, pericardial oedema, heart rate, eyes pigmentation and skeletal deformities (head, tail, vertebral, column) – and 75% epiboly, mortality rate, hatching success, larvae length, mouth opening and jaw deformities NOEC	96 h-NOEC: 3.8 124 hpf – NOEC < 1.2	McGowan et al. (2013)
<i>Cyclohexylbenzene</i> Chordata – fish	Fathead minnow (FW) <i>Pimephales promelas</i> Turbot (SW) <i>Scophthalmus maximus</i>	96 h Eggs Exposure type: 9 day exposure period, in four sampling points: 53 h postfertilization (hpf) (day 2) – 75% epiboly; 100 hpf (day 4) – before hatching (108 hpf)-after heart start beating (92 hpf); 124 hpf (day 5) – after hatching (108 hpf); and 220 hpf (day 9) – after mouth opening (204 hpf) [P] 0.128–5.00 mg/L	Mortality LC ₅₀ Embryo malformations – developmental delay, abnormal cellular masses, yolk sac alterations, oil globule fragmentation, oil globule position, no rupture of egg membrane, pericardial oedema, heart rate, eyes pigmentation and skeletal deformities (head, tail, vertebral, column) – and 75% epiboly, mortality rate, hatching success, larvae length, mouth opening and jaw deformities NOEC	96 h-LC ₅₀ : 0.103 124 hpf – NOEC < 5.0	Eastman (2013) McGowan et al. (2013)
<i>Hexane</i> Chordata – fish	Fathead minnow (FW) <i>Pimephales promelas</i> Yellowtail flounder (SW) <i>Pleuronectes ferrugineus</i> Turbot (SW) <i>Scophthalmus maximus</i>	Organisms with 31 days, 20.4 mm, 0.123 g Exposure type: F 96 h Organisms of 1 year, 9.5–12.3 cm, 13.64–33.30 g) Exposure type: F 14 days [P] 3.03 mL/g food Eggs Exposure type: 9 day exposure period, in four sampling points: 53 h postfertilization (hpf) (day 2) – 75% epiboly; 100 hpf (day 4) – before hatching (108 hpf)-after heart start beating (92 hpf); 124 hpf (day 5) – after hatching (108 hpf); and 220 hpf (day 9) – after mouth opening (204 hpf) [P] 0.179–7.0 mg/L	Mortality LC ₅₀ Histochemical and biochemical effects (hepatosomatic index, total and neutral lipid content, hydrocarbons, steryl esters, wax esters, ketones, triacylglycerols, free fatty acids, alcohols, sterols, diacylglycerols, acetone-mobile polar lipids and phospholipids percentage of liver lipids) Embryo malformations – developmental delay, abnormal cellular masses, yolk sac alterations, oil globule fragmentation, oil globule position, no rupture of egg membrane, pericardial oedema, heart rate, eyes pigmentation and skeletal deformities (head, tail, vertebral, column) – and 75% epiboly, mortality rate, hatching success, larvae length, mouth opening and jaw deformities NOEC, LOEC	96 h-LC ₅₀ : 2.5 (2.1–2.98) 14 d-NOEC < 3.03 124 hpf – NOEC: 2.8 124 hpf – NOEC: 7.0	ECOTOX (2014b) and references therein Scott et al. (2002) McGowan et al. (2013)

Isononanol

Chordata – fish	Goldfish (FW) <i>Carassius auratus</i> Japanese medaka (FW) <i>Oryzias latipes</i>	Exposure type: S 24 h	Mortality LC ₅₀	24 h-LC ₅₀ : 16	OECD-SIDS (2002b) and references therein OECD-SIDS (2002b) and references therein
		Organisms of 17–19 mm and 0.063–0.11 g Exposure type: R 24 h [P] 7.9–40.0 mg/L	Mortality LC ₅₀	24 h-LC ₅₀ : 27.7 (16.6–37.1)	
		Exposure type: F 14 days Organisms of 18–21 mm and 0.082–0.14 g Exposure type: F 14/15 days [P] 0.512–20.0 mg/L	Mortality LC ₅₀ Behavioural (feeding behaviour) (behaviour change was observed, most frequently on the 3rd day of exposure) EC ₅₀ , NOEC Mortality LC ₅₀	14 d-LC ₅₀ > 20 14 d-EC ₅₀ : 3.2 (2.17–4.72) 14 d-NOEC: 1.28	
	Fathead minnow (FW) <i>Pimephales promelas</i>	96 h	Mortality LC ₅₀	96 h-LC ₅₀ : 5.7	OECD-SIDS (2002b) and references therein
Trichloroethylene Chordata – Amphibia	African clawed frog (FW) <i>Xenopus laevis</i>	Embryos Exposure type: R 96 h	Physiological effects (mixed-function oxidase or epoxide hydrolase activity) Mortality Growth Teratological effects	96 h-EC ₅₀ : 36 (27–49) 96 h-LC ₅₀ : 434.9 (381.0–293.0) 96 h-LOEC: 29 Gut miscoiling and microphthalmia 96 h-LOEC > 15 Abnormal mouth development and muscular kinking 96 h-LOEC: >40 Hypognathia 96 h-LOEC: >300	Fort et al. (1993)
	Wood frog (FW) <i>Rana sylvatica</i> Green frog (FW) <i>Rana clamitans</i> American toad (FW) <i>Bufo americanus</i> Spotted salamanders (FW) <i>Ambystoma maculatum</i>	Larvae Exposure type: SS 96 h [P] 12.5–85 mg/L	Deformations (development of tadpoles) EC ₅₀ Motionless LOEC	Wood frog 96 h-EC ₅₀ : 45.7 (37.8–59.7) (nc) Wood frog 96 h-EC ₅₀ : 32.2 (25.1–40.1) (mc) Green frog 96 h-EC ₅₀ : 33.6 (25.3–47.1) (nc) Green frog 96 h-EC ₅₀ : 22 (16.1–30.2) (mc) American toad 96 h-EC ₅₀ : >85 Spotted salamander 96 h-EC ₅₀ : 60 (nc) Spotted salamander 96 h-EC ₅₀ : 40 (mc)	McDaniel et al. (2004)
Chordata – fish	Zebrafish (FW) <i>Danio rerio</i>	Exposure type: S 14 days	Behavioural effects (not specified) NOEC	Motionless 96 h-LOEC: 60 14 d-NOEC: 3.1	ECOTOX (2014) and references therein
TRICHLOROETHYLENE Chordata – fish	Flagfish (FW) <i>Jordanella floridae</i>	Juveniles (2–4 months) Exposure type: F 12, 24, 36, 48, 72, 96 h Exposure type: S 96 h	Mortality LC ₅₀	Flow-through exposure 12 h-LC ₅₀ 29.46 24 h-LC ₅₀ 29.46 36 h-LC ₅₀ 29.46 48 h-LC ₅₀ 29.46 72 h-LC ₅₀ 29.46 96 h-LC ₅₀ 28.28 (26.1–30.2) Semi-static exposure 96 h-LC ₅₀ 63.1 (53.3–74.4)	Smith et al. (1991)
	Embryo/larval fish (10 d) Week old fry (28 d)	Exposure type: F	Mortality Growth LOEC	Mortality 10 d-LOEC: 11 Egg hatchability 10 d-LOEC: 21.2 Mortality 28 d-LOEC 14.85	Smith et al. (1991)

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Table 3 (continued)

Species group	Common name Scientific name ^a	Experimental specifications ^b	Toxicological effect measured Test endpoint ^c	Toxic concentration (mg/L) ^d	References
	Bluegill (FW) <i>Lepomis macrochirus</i>	10 and 28 days Exposure type: F 2 h [P] 0.100 mg/L	Physiological effect (ventilation) NOEC	Growth 28 d-LOEC > 20.9 2 h-NOEC < 0.100	Diamond et al. (1990)
	Rainbow trout (FW) <i>Oncorhynchus mykiss</i>	Fingerlings (4 cm, 2–4 g) Exposure type: F 1 h Exposure type: F 21 days	Physiological effects (ventilatory rates and general activity of the exposed fish) NOEC Physiological effect (respiration) Biochemical effects (haematological parameters) Enzymatic activity Feeding behaviour NOEC	1 h-NOEC < 0.01 21 d-NOEC < 0.0066	Kaiser et al. (1995) ECOTOX (2014) and references therein
	Japanese medaka (FW) <i>Oryzias latipes</i>	Post-hatched organisms of Japanese medaka (JM): 4, 7, 10, 13, 16, 28, 42, 180 days Exposure type: F 7 days	Mortality LC ₅₀	4 d-JM 7 d-LC ₅₀ : 37.0 (29.9–44.4) 7 d-JM 7 d-LC ₅₀ : 32.5 (30.0–35.3) 10 d-JM 7 d-LC ₅₀ : 25.2 (19.7–31.0) 13 d-JM 7 d-LC ₅₀ : 24.2 (19.2–29.5) 16 d-JM 7 d-LC ₅₀ : 23.0 (21.6–24.4) 28 d-JM 7 d-LC ₅₀ : 26.7 (21.0–31.5) 42 d-JM 7 d-LC ₅₀ : 25.7 180 d-JM 7 d-LC ₅₀ : 25.8	Manning et al. (2001)
	Rosy bitterling (FW) <i>Rhodeus ocellatus ocellatus</i>	Eggs/embryos Exposure type: S 24 h	Genotoxicity (micronucleus assays) NOEC	24 h-NOEC < 3	Hayashi et al. (1998)
	Turbot (SW) <i>Scophthalmus maximus</i>	Eggs Exposure type: 9 days exposure period, in four sampling points: 53 h postfertilization (hpf) (day 2) – 75% epiboly; 100 hpf (day 4) – before hatching (108 hpf)–after heart start beating (92 hpf); 124 hpf (day 5) – after hatching (108 hpf); and 220 hpf (day 9) – after mouth opening (204 hpf) [P] 0.230–9.0 mg/L	Embryo malformations – developmental delay, abnormal cellular masses, yolk sac alterations, oil globule fragmentation, oil globule position, no rupture of egg membrane, pericardial oedema, heart rate, eyes pigmentation and skeletal deformities (head, tail, vertebral, column) – and 75% epiboly, mortality rate, hatching success, larvae length, mouth opening and jaw deformities NOEC	124 hpf – NOEC < 9.0	McGowan et al. (2013)
XYLENE					
Chordata – Amphibia	Leopard frog (FW) <i>Rana pipiens</i>	28 days m-Xylene	Effect on hatching LC ₅₀	28 d-EC20: 0.31 28 d-LC ₅₀ : 2.3	Nagpal (2007) and references therein
Chordata – fish	Ray-finned fish (FW) <i>Bryconamericus iheringii</i>	Organisms with 4.7 cm and 2.85 g Exposure type: SS 1–96 h m-, p-, o-Xylene	Mortality LC ₅₀	m-Xylene 96 h-LC ₅₀ : 11.23 p-Xylene 96 h-LC ₅₀ : 6.90 o-Xylene 96 h-LC ₅₀ : 9.94	Di Marzio and Saenz (2004)
	Ten spotted live-bearer (FW) <i>Cnesterodon decemmaculatus</i>	Exposure type: S 96 h m-, p-, o-Xylene	Mortality LC ₅₀	96 h-LC ₅₀ : 6–11	Di Marzio et al. (2001)
	Fathead minnow (FW) <i>Pimephales promelas</i>	Exposure type: S Exposure type: F 96 h	Mortality LC ₅₀	Static 96 h-LC ₅₀ : 16.4 Flow-through 96 h-LC ₅₀ : 16 (14.3–18)	ECOTOX (2014c) and references therein
	Japanese medaka (FW) <i>Oryzias latipes</i>	Exposure type: SS 48 and 96 h m-xylene	Mortality LC ₅₀	96 h-LC ₅₀ : 32	Yoshioka and Ose (1993)
	Tilapia (FW) <i>Tilapia zillii</i>	Exposure type: S 96 h	Mortality, LC ₅₀ Histological effects, EC ₅₀ Feeding behaviour, EC ₅₀	96 h-LC ₅₀ : 39.8 (36.5–42.7) 96 h-EC ₅₀ : 13.93	ECOTOX (2014c) and references therein

Nominal concentration (nc), measured concentration (mc). Hour (h), days (d), week (w). n.a. – not available.

^a Freshwater (FW); seawater (SW).

^b Static (S), semi-static (SS), flow-through (F), ex-situ bioassay (E), [P] – concentration of the pollutant.

^c Effective concentration for x% of tested organisms (EC_x), lethal concentration for 50% of tested organisms (LC₅₀), inhibition concentration for 50% of tested organisms (IC₅₀), no observable effect concentration (NOEC), lowest observable effect concentration (LOEC).

^d 95% confident limits of LC₅₀, EC₅₀, IC₅₀, NOEC and LOEC were inserted between brackets whenever possible.

Table 4

List of species exposed to the selected hazardous and noxious substances (HNSs), acrylonitrile, butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene, in the reviewed literature (X indicates the HNS to which each species was exposed).

	Habitat ^a	Acrylonitrile	N-butyl acrylate	Cyclohexylbenzene	Hexane	Isononanol	Trichloroethylene	Xylene
Bacterium – <i>Photobacterium phosphoreum</i>	SW							X
Ciliate protozoa – <i>Tetrahymena pyriformis</i>	FW							X
Green alga – <i>Scenedesmus subspicatus</i>	FW	X					X	
Green alga – <i>Desmodesmus subspicatus</i>	FW		X				X	
Green alga – <i>Selenastrum capricornutum</i>	FW		X			X	X	X
Green alga – <i>Chlorella kessleri</i> , <i>Desmodesmus quadricauda</i>	FW						X	
Green alga – <i>Chlorococcales</i>	FW						X	
Green alga – <i>Scenedesmus quadricauda</i>	FW						X	X
Green alga – <i>Chlorella vulgaris</i>	FW						X	
Green alga – <i>Chlamydomonas reinhardtii</i>	FW						X	X
Green alga – <i>Chlorella ellipsoidea</i> , <i>Chlorococcum</i> sp., <i>Gleocystis ampla</i> , <i>Nannochloris</i> sp., <i>Scenedesmus obliquus</i> , <i>Tetraselmis</i> sp.	SW						X	
Diatoms – <i>Cyclotella</i> sp., <i>Cylindrotheca</i> sp., <i>Navicula saprophila</i> sp., <i>Nitzschia dissipata</i> , <i>Nitzschia pusilla</i> sp., <i>Thalassiosira weissflogii</i>	FW						X	
Diatom – <i>Skeletonema costatum</i>	SW	X						
Cyanobacteria – <i>Synechococcus elongatus</i> f. <i>thermalis</i> , <i>Synechococcus leopoliensis</i> , and <i>Microcystis aeruginosa</i>	FW						X	
Phytoplankton	FW						X	
Macroalga – <i>Fucus vesiculosus</i>	SW		X					
Duckweed – <i>Lemna minor</i>	FW	X						
Water-celery, tapegrass – <i>Vallisneria americana</i>	FW						X	
Rotifer – <i>Brachionus calyciflorus</i>	FW				X			X
Rotifer – <i>Brachionus plicatilis</i>	SW				X			X
Rotifera	FW						X	
Tubificid worm, Oligochaete – <i>Limnodrilus hoffmeisteri</i>	FW	X						
Marine bristle worms – <i>Pomatoceros triqueter</i>	SW		X					
Snail – <i>Amphimelania holandri</i> Fér.	FW							X
Snail – <i>Lymnaea stagnalis</i> L.	FW							X
Mollusc – <i>Radix plicatula</i>	FW	X						
Zebra mussel – <i>Dreissena polymorpha</i>	FW						X	
Clam – <i>Gafrarium divaricatum</i>	SW							X
Clam – <i>Corbicula fluminea</i>	FW						X	
Midge – <i>Chironomus</i> sp.	FW	X						
Amphipod – <i>Hyalella curvispina</i>	FW							X
Amphipod – <i>Gammarus locusta</i>	SW							X
Copepod – <i>Tisbe battagliai</i>	SW		X					
Cyclopoid copepod – <i>Cyclops</i> sp.	FW						X	
Water flea – <i>Daphnia magna</i>	FW	X	X			X	X	X
Water flea – <i>Daphnia spinulata</i>	FW							X
Water flea – <i>Ceriodaphnia dubia</i>	FW							X
Water flea – <i>Daphnia pulex</i>	FW			X				
Isopod – <i>Asellus aquaticus</i> L.	FW							X
Amphipod – <i>Gammarus fossarum</i>	FW							X
Fairy shrimp – <i>Streptocephalus proboscideus</i>	SW							X
Brine shrimp – <i>Artemia salina</i>	SW	X						X
Sea urchin – <i>Paracentrotus lividus</i>	SW		X	X				
Toad – <i>Bufo bufo</i> ssp. <i>gargarizans</i>	FW	X						
Wood frog – <i>Rana sylvatica</i>	FW						X	
Green frog – <i>Rana clamitans</i>	FW						X	
American toad – <i>Bufo americanus</i>	FW						X	
Spotted salamanders – <i>Ambystoma maculatum</i>	FW						X	
African clawed frog – <i>Xenopus laevis</i>	FW						X	
Leopard frog – <i>Rana pipiens</i>	FW							X
Grass carp, white mur – <i>Ctenopharyngodon idella</i>	FW	X						
Common carp – <i>Cyprinus carpio</i>	SW	X						
Sheepshead minnow – <i>Cyprinodon variegatus</i>	SW	X	X					
European seabass – <i>Dicentrarchus labrax</i>	SW	X						
Rainbow trout – <i>Oncorhynchus mykiss</i>	FW	X	X				X	
Japanese medaka – <i>Oryzias latipes</i>	FW	X				X	X	X
Guppy – <i>Poecilia reticulata</i>	FW	X						
Fathead minnow – <i>Pimephales promelas</i>	FW		X	X	X			X
Turbot – <i>Scophthalmus maximus</i>	SW		X	X				
Yellowtail flounder – <i>Pleuronectes ferrugineus</i>	SW				X			
Goldfish – <i>Carassius auratus</i>	FW					X		
Zebrafish – <i>Danio rerio</i>	FW						X	
Flagfish – <i>Jordanella floridae</i>	FW						X	
Bluegill – <i>Lepomis macrochirus</i>	FW						X	
Rosy bitterling – <i>Rhodeus ocellatus ocellatus</i>	FW						X	
Ray-finned fish – <i>Bryconamericus iheringii</i>	FW							X
Ten spotted live-bearer – <i>Cnesterodon decemmaculatus</i>	FW							X
Tilapia – <i>Tilapia zillii</i>	FW							X

^a Freshwater (FW), Seawater (SW).

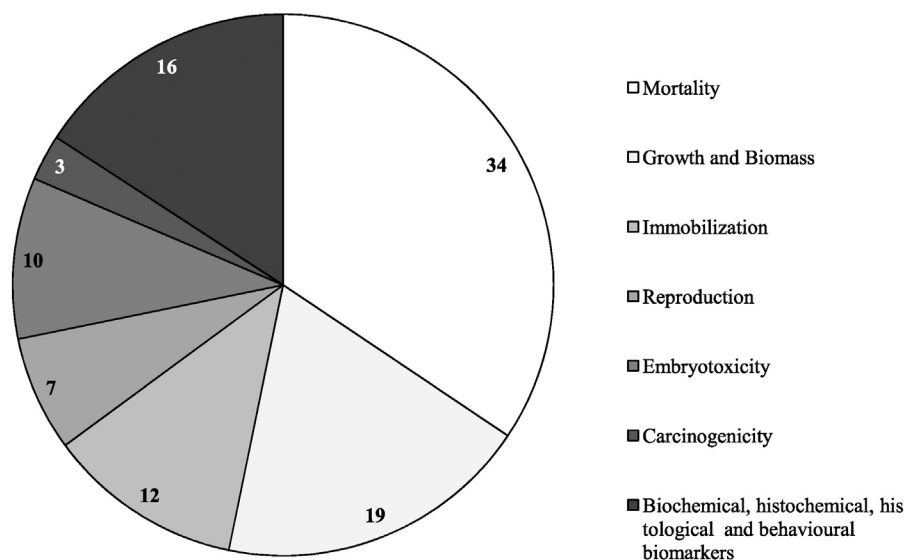


Fig. 1. Main endpoints evaluated in the literature reviewed (in percentage). The revision led to the identification of 121 bioassays carried out, from 1990 onward, with the HNS of interest and aquatic organisms (68 different species). Bioassays were carried out mainly for freshwater organisms (51 species) although some works with marine organisms have been found (17 species).

detoxification (CBCSS-NRC, 2003). However, more toxic products can also be produced in this process. In such cases, the measured ecotoxicological effects can be due not only to the HNS itself but also to its hazardous metabolites. Acrylonitrile, on its own, is recognised as a central nervous system depressant (Olson, 2007). Nevertheless, bioassays with mammals showed that the metabolism of acrylonitrile can result in the production of reactive epoxides of 2-cyanoethylene oxide and cyanide which can also induce toxicity in the brain (Lech et al., 1996; Long et al., 2001; Kirman et al., 2005). The same can happen for hexane: the neurotoxic metabolite 2,5-hexanedione is responsible for hexane toxicity (ATSDR, 1999). In addition, during the biotransformation of trichloroethylene, several reactive intermediate metabolites are produced and known to induce oxidative stress (Vidal et al., 2001). Examples of these are chloral hydrate, trichloroacetate and dichloroacetate which were shown to cause liver tumours in mouse (Barton et al., 1994), and trichloroethylene epoxide, which is highly embryotoxic and possibly involved on developmental toxicity in rats, rabbits and mouse (Fort et al., 1993). Table 5 presents a list of metabolites reported in organisms exposed to the selected HNS discussed in this review. Further research about their single effects on organisms will bring insight on mechanisms of toxicity of parent compounds.

Xenobiotic contaminants are generally known to cause oxidative stress through either the production of ROS during detoxification reactions or the direct reaction of the chemicals with cellular macromolecules. Reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl, alkoxy and peroxy radicals, can react with proteins, lipids and nucleic acids, modifying their chemical form and hampering their functions. Hazardous and noxious substances are apparently no exception in regard to ROS production and generation of oxidative stress. Reports of increased ROS generation due to exposure to acrylonitrile in rats (Pu et al., 2009) and trichloroethylene in an aquatic species (Asian clam (Vidal et al., 2001)) are available in the literature. HNS and their reactive metabolites are also recognised to bind to macromolecules with important roles in cellular function, e.g. compounds with sulfhydryl (SH) groups (GSH), DNA, RNA and proteins. This was observed for acrylonitrile (Farooqui and Ahmed, 1983; Kirman et al., 2005), n-butyl acrylate (Wiegand et al., 1989; Tyler et al., 1993; Freidig, 2000), hexane (ATSDR, 1999), trichloroethylene (Kaneko et al., 1997; Cai and Guengerich, 2000; Vidal et al., 2001) and xylene (ATSDR, 2007). For example, acrylonitrile was able to bind to parvalbumin in trout's muscle, probably through the amino acid histidine (Lech et al., 1996). Parvalbumin is a Ca^{2+} binding protein involved

in the regulation of calcium levels in various parts of the body, ranging from neurons to fast-twitch muscles. The binding to macromolecules induces alterations in cells also contributing for oxidative stress. Moreover, the depletion of GSH and increase in DNA oxidative damage have been found in rats exposed to acrylonitrile (Silver et al., 1982; Kirman et al., 2005; Pu et al., 2009), n-butyl acrylate (Freidig, 2000), trichloroethylene (Kaneko et al., 1997), xylene (Fishbein, 1985) and a marine fish (seabass) exposed to acrylonitrile (Neuparth et al., 2013). Furthermore, increased lipid peroxidation was also found for acrylonitrile in rats (Esmat et al., 2007), trichloroethylene in Asian clam (Vidal et al., 2001) and xylene in porcine proximal tubular cells (Al-Ghamdi et al., 2003a).

As a result of the induced stress, cells respond by triggering antioxidant protective mechanisms. A cooperative effort between antioxidant enzymes and endogenous antioxidant molecules (e.g. GSH, tocopherols) protects cells against oxidative damage (Abele et al., 2011; Farooqui and Farooqui, 2012) by neutralising ROS inside the cell. Among antioxidant enzymes, GST, CAT, SOD, glutathione peroxidase (GPx) and glutathione reductase (GR) are relevant components of the cell defence system. Therefore, the activity of these enzymes, the levels of endogenous antioxidant molecules and the detection of oxidative damage to cellular macromolecules are widely used as biomarkers in environmental monitoring (Ferreira et al., 2005; Galhano et al., 2011). Exposure to HNS can either trigger or inhibit the activities of antioxidant enzymes. For mammals, several are the reports indicating the induction of antioxidant enzymatic activity brought about by the selected HNS (Goel et al., 1992; Kaneko et al., 1997; Jiang et al., 1998). Similar antioxidant mechanisms seemed to be activated in aquatic organisms. For instance, acrylonitrile induced the activity of CAT and GST but inhibited SOD activity in seabass' liver after a 15-day exposure (Neuparth et al., 2013). The authors hypothesised that the SOD inhibition could be a consequence of decreased availability of copper and zinc, essential for its activity, since acrylonitrile was shown to form stable complexes with these metals. Still, the results suggested the involvement of these enzymes in the protection of the organism against oxidative damage. This was further supported by the lack of differences in lipid peroxidation levels detected for exposed and control fish (Neuparth et al., 2013). Trichloroethylene-treated freshwater clams also presented increased activity of CAT and increased levels of lipid peroxidation but only for the lowest concentrations tested (1.2 and 6.25 mg/L (Vidal et al., 2001)). For higher exposure concentrations (25 and 100 mg/L), no significant changes relative to controls were observed for both biomarkers.

Table 5

Metabolites derived from the biotransformation of hazardous and noxious substances (HNSs) found in the urine, blood or tissues of organisms (mainly mammals) previously exposed to HNS.

HNS	Metabolites	References
Acrylonitrile	2-Cyanoethylene oxide (CEO), vinyl chloride, acrylamide, 1,3-butadiene, <i>N</i> -acetyl- <i>S</i> -(2-cyanoethyl)- <i>L</i> -cysteine, <i>N</i> -acetyl- <i>S</i> -(2-hydroxyethyl)- <i>L</i> -cysteine, 4-acetyl-3-carboxy-5-cyanotetrahydro-1,4-2H-thiazine, cyanoacetic acid, hydroxypropionitrile, cyanide, thiocyanate	Bolt and Lewalter (1993), Lech et al. (1996), Kirman et al. (2005)
<i>N</i> -butyl acrylate Cyclohexylbenzene	Acrylic acid, butyl alcohol, mercapturic acid derivatives (e.g. <i>N</i> -acetyl- <i>S</i> -(2-carboxyethyl)cysteine and its sulfoxide) Glucosiduronic acid, 2-hydroxybiphenyl, 3-hydroxybiphenyl, 4-hydroxybiphenyl, 4-phenyl-catechol, phenols, ether sulphates, 4-phenylphenylmercapturic acid	Tyler et al. (1993), IARC (1999) PubChem (2014)
Hexane	1-Hexanol, 3-hexanol, hexanoic acid, 2-hexanol, 2-hexanone, 2,5-hexanediol, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone, 2,5-hexanedione, 2,5-dimethylfuran, gammavalerolactone	ATSDR (1999), USEPA (2005)
Isononanol Trichloroethylene	Alcohols derivatives, ketones derivatives, aldehydes derivatives, carboxylic acid derivatives Choral, trichloroethanol, trichloroethanol glucuronide, trichloroacetic acid, dichloroacetic acid, oxalic acid, glycolic acid, dichlorovinyl-cysteine (DCVC), <i>S</i> -(1,2-dichlorovinyl)glutathione, <i>S</i> -(1,2-dichlorovinyl)- <i>L</i> -cysteine, trichloroethylene oxide, chloroform	EFSA (2012), SCBT (2014) Fort et al. (1993), Barton et al. (1994), Vidal et al. (2001)
Xylene	Methylhippotic acids, <i>o</i> -methylbenzylmercapturic acid, xyleneols, glucuronic acid conjugates, arene oxides, methylbenzaldehyde, methylbenzoic acid	Fishbein (1985), ATSDR (2007)

A possible explanation for this is related to the ability of trichloroethylene to complex with oxygenated cytochrome P450 previously described in mammals, which can cause the inactivation of cytochrome P450, therefore, limiting the production of reactive metabolites (Miller and Guengerich, 1982). Hence, at higher test concentrations, in the absence of reactive metabolites, the activity of antioxidant enzymes and the extent of lipid peroxidation would not be altered (Vidal et al., 2001). The activity of GST and CAT was significantly induced by xylene in day 36 followed by a significant drop after 50 days of exposure in brine shrimp (*Gammarus locusta*) (Neuparth et al., 2011a). Alterations on SOD activity were observed as well: the enzyme activity increased over the first two sampling periods (days 36 and 50). At day 63, the activity of those antioxidant enzymes returned to control values.

Increased and decreased activities of other enzymes also important for cell functioning were additionally recorded in mammals. For instance, carboxylase and cytosolic dehydrogenase enzymes were shown to also contribute for HNS metabolism, namely, the hydrolysis of *n*-butyl acrylate (Tyler et al., 1993) and the oxidation of isononanol and xylene (Fishbein, 1985; EFSA, 2012) respectively. In addition, in studies with CYP2E1-Null and wild type mice, rhodanese was shown to have a prominent role in diminishing the toxic action of a metabolite of acrylonitrile (cyanide) by converting it into thiocyanate (Wang et al., 2002). Other enzymes associated with important cell metabolic pathways, such as carbohydrates (e.g. beta-glucuronidase, sorbitol dehydrogenase) and pentose phosphate (e.g. glucose-6-phosphate dehydrogenase), signal transduction pathways (e.g. acid and alkaline phosphatases) and oxidative stress and damage (e.g. glucose-6-phosphate dehydrogenase, lactate dehydrogenase (LDH), aspartate transaminase, alanine transaminase) had their activities changed after HNS exposure. More precisely, increased activities of glucose-6-phosphate dehydrogenase, acid and alkaline phosphatase, beta-glucuronidase and LDH were recorded in liver and lungs of mammals exposed to hexane (EHC, 1991). The same was observed for serum activities of sorbitol dehydrogenase and transaminase in rat livers exposed to acrylonitrile (Silver et al., 1982). Furthermore, cyclohexylbenzene induced alterations in serum activities of aspartate transaminase (increased and decreased), alanine transaminase (increased) and LDH (increased) in treated mice (PubChem, 2014).

5.2. Cell injury and haematological parameters

Cell injury can result from HNS exposure. Many of the previously mentioned enzymes are in fact used to identify the location and severity of tissue damage (e.g. glucose-6-phosphate dehydrogenase, beta-glucuronidase, LDH, sorbitol dehydrogenase and transaminase). Apart from beta-glucuronidase, which is used as an indirect marker of inflammation and an index of tissue injury in muscle, the remaining enzymes are good indicators of liver, as well as other organs, health (PubChem,

2014). Alteration of these enzymes activity can thus constitute a helpful indicator of HNS contamination. Other parameters can be relevant for the assessment of cell injury, for instance, activity of enzymes with a central role in the apoptosis process and the disturbance of cell viability and functioning. Caspase-3, a member of the cysteine-aspartic acid protease family with a central role in the execution-phase of cell apoptosis, was activated in renal proximal tubular epithelial cells derived from pig and caused cell injury after exposure to xylene (Al-Ghamdi et al., 2003b). The assessment of cell membrane integrity can also be a suitable indicator of cell injury. For instance, by assessing LDH leakage and trypan blue exclusion test of cell viability, Esmat et al. (2007) observed that acrylonitrile caused a 50% membrane damage to primary rat glial cells. Moreover, membrane properties (e.g. permeability, potential, impedance, fluidity, thickness, surface tension, etc.) were compromised after exposure to cyclohexylbenzene in mammals (PubChem, 2014), hexane in the squid *Loligo forbesi* (Haydon and Urban, 1983) and trichloroethylene in the green algae *Chlamydomonas reinhardtii* (Brack and Frank, 1998).

Effects on blood chemistry and haematological parameters were also observed for example in mammals exposed to cyclohexylbenzene (PubChem, 2014) and isononanol (OECD-SIDS, 2002b). This may possibly be due to the ability of some HNS and their metabolites to directly bind to haemoglobin as described for acrylonitrile (Farooqui and Ahmed, 1983; Lech et al., 1996), hexane (ATSDR, 1999) and xylene (ATSDR, 2007). Acrylonitrile was inclusively observed to cause increased susceptibility to osmotic fragility and haemolysis of erythrocytes in rats (Farooqui and Ahmed, 1983). The authors inferred that this binding may constitute an ecotoxicological risk if the cellular function of the protein is affected by conformational changes. These parameters may also constitute relevant and less invasive biomarkers of HNS contamination especially for marine vertebrates.

5.3. HNS carcinogenicity

Hazardous and noxious substances are known to bind to DNA and RNA with evidence of increased DNA strand breaks having been found for acrylonitrile (Farooqui and Ahmed, 1983; Kirman et al., 2005), *n*-butyl acrylate (Wiegand et al., 1989; Tyler et al., 1993; Freidig, 2000), hexane (ATSDR, 1999), trichloroethylene (Kaneko et al., 1997; Cai and Guengerich, 2000; Vidal et al., 2001) and xylene (ATSDR, 2007). This binding can trigger a cascade of events that can affect the functioning of cells and organs which can potentiate the development of cancer (Farooqui and Farooqui, 2012).

Acrylonitrile is classified as probably carcinogenic to human, under the International Agency for Research on Cancer (IARC) (Group 2B) and EPA classification system (Group B1) (IARC, 1999; USEPA, 1999). Acrylonitrile and some of its metabolites already mentioned (e.g. vinyl chloride, acrylamide, 1,3-butadiene (Long et al., 2001; Kirman et al.,

2005)) are recognised as carcinogenic to mammals, mainly targeting stomach and brain (Farooqui and Ahmed, 1983; Long et al., 2001; Carrera et al., 2007; Olson, 2007; Pu et al., 2009). This HNS was also shown to cause oxidative damage to DNA in rats (Kirman et al., 2005) which could be associated to the formation of acrylonitrile-induced brain tumours (Pu et al., 2009). The carcinogenic potential of acrylonitrile was shown to be dependent upon the species, type of tumours and the length of follow up (Léonard et al., 1999). In fact, in bioassays carried out with Japanese medaka and guppy, acrylonitrile showed signs of cytotoxicity but no evidence of carcinogenicity to both fish species (Hawkins, 1991; Ortego et al., 1996). Proliferation of hepatocytes was detected, as assessed by proliferating cell nuclear antigen immunohistochemistry, but again no correlation with subsequent carcinogenesis was found.

Trichloroethylene was also included in Group 2A of IARC classification system (IARC, 1995). On the other hand, EPA does not have a consensus classification for its carcinogenicity (USEPA, 2001) although data indicate this HNS is a likely human carcinogen. In fact, structural chromosomal aberrations and micronuclei were observed in the cells of rose bitterling (*Rhodeus ocellatus*) embryos grown in water containing trichloroethylene (Hayashi et al., 1998). The carcinogenicity of trichloroethylene is thought to reside in its reactive metabolites (Davidson and Beliles, 1991), according to evidence of their mutagenic and tumorigenic action reported in mammals (Barton et al., 1994; Kaneko et al., 1997).

Xylene could not be classified as to its carcinogenicity to humans being thus placed in Group 3 and Group D of IARC and EPA classification systems, respectively (ATSDR, 1999). Hexane was also included in EPA Group D but not listed in IARC carcinogenicity ratings (IARC, 1989). Xylene was found to be embryotoxic and teratogenic to rats, affecting especially the brain, liver, lungs and heart (Fishbein, 1985) despite no significant chromosomal abnormalities or mutagenicity being found. Hexane appeared not to cause embryotoxic, fetotoxic, teratogenic or carcinogenic effects in some mammals (e.g. rats, mice) (EHC, 1991). Though, increased levels of plasma enzymes (glucose-6-phosphate dehydrogenase and LDH), liver weight and hepatic microsomal protein in mammals were indicative of liver damage after hexane exposure (EHC, 1991).

For n-butyl acrylate, cyclohexylbenzene and isononanol, no carcinogenicity classification category was yet assigned. Cyclohexylbenzene was shown to increase incidence of liver, bladder and kidney tumours in mice (PubChem, 2014) but straightforward conclusions have not been achieved due to the scarcity of information. For n-butyl acrylate, no mutagenic and cytotoxic effects were found in Ames test, a biological assay to assess the mutagenic potential of chemical compounds (OECD-SIDS, 2002a). Furthermore, occurrence of epidermal tumours (DePass et al., 1984; Wiegand et al., 1989), neoplastic changes after inhalation (Wiegand et al., 1989) or evidence of teratogenic responses (IARC, 1985) were not detected in mice and rats. Indication that isononanol induced no embryotoxic, fetotoxic or teratogenic effects in mammals was also found in literature (ExxonMobil, 2006; EFSA, 2012). These reports also indicate that mutagenic effects could not be detected in the Ames test (ExxonMobil, 2006; EFSA, 2012), as well as, structural chromosomal aberrations, including polyploid CHL/IU cells (OECD-SIDS, 2002b). Data suggest that n-butyl acrylate, cyclohexylbenzene and isononanol may have a low carcinogenic risk.

The existing body of evidence about the potential carcinogenicity of the seven selected HNSs is still limited and focused on mammals. Taking into consideration the potential to induce oxidative DNA damage and the repercussions of an increased cancer incidence due to HNS exposure, further research is necessary to clarify their carcinogenicity potential to marine organisms.

5.4. Neurotoxic and endocrine alterations

Neurotoxic effects possibly triggered by the selected HNS are still little understood. Nevertheless, the brain was identified as target site for

many of these HNSs. Acrylonitrile, its metabolites and the toxic metabolite of hexane, 2,5 – hexanedione, have been described as brain neurotoxicants (ATSDR, 1999; Olson, 2007). In addition, exposure to trichloroethylene can also result, among others, in neurotoxicity (including changes observed in the central and autonomic nervous system in humans) (Kaneko et al., 1997). Alterations in levels of neurotransmitters and lipid composition of the brain after exposure to xylene were observed for rats and humans (ATSDR, 2007). Parameters suitable for the assessment of the neurotoxicity associated with the selected HNS would be therefore useful to better understand their mode of action in the nervous system. For instance, the activity of cholinesterase enzymes has been frequently used as biomarkers in aquatic organisms for this purpose (Yuanqing et al., 2013; Rodrigues et al., 2014). In mammals, evidence that some HNS can interfere with the activity of these enzymes has been previously published. For instance, acetylcholinesterase (AChE) activity in both mouse blood and brain was affected in a hormetic manner by acrylonitrile (Yuanqing et al., 2013). Experiments with swine tracheal smooth muscle showed that trichloroethylene decreased the activity of AChE in epithelia (Chen et al., 2005). Pseudocholinesterases may also be affected. For example, plasma butyrylcholinesterase increased twofold in trichloroethylene-treated male mice (Kanje et al., 1981). In addition, inhibition of human serum cholinesterase in vitro was also verified after exposure to a distillation residue of hexane (Vilanova and Vicedo, 1983). The measurement of the activity of these enzymes should therefore be used in further research as previously suggested by Neuparth et al. (2013). The authors inferred that the alterations noted on the swimming performance of seabass exposed to acrylonitrile could be linked to effects on nervous systems.

Similarly, scarce evidence that HNS can affect endocrine system can be found in literature. Once more, available information concerns mammals and data produced until now remains controversial. Early works suggested that trichloroethylene induced neither significant effects in adrenal glands of rabbits nor histological changes in exposed mice (EURAR (2004b) and references therein). However, studies reporting its toxicity to adrenal glands of rabbits and in reproductive systems of mice have also been published (Verma and Rana (2009) and references therein). Evidence that this HNS can inclusively alter endocrine functions and fertility in humans was also put forward (USEPA, 2012). Trichloroethylene and xylene are included in the Endocrine Disruptor Screening Programme (EDSP) of EPA as substances that should be candidates at least for screening purposes. Xylene is also a candidate substance to the European Community Rolling Action Plan (CoRAP): not particularly for its potential as an endocrine disruptor but for its possible reproductive toxicity, developmental neurotoxicity and wide disperse and consumer use (CoRAP, 2013a). This HNS was shown not to cause adverse adrenal, thyroid and parathyroid effects in dogs and rats after its inhalation (ATSDR, 2007). N-butyl acrylate was also included in CoRAP due to the lack of information regarding its reproduction toxicity (CoRAP, 2013b). There is evidence that this HNS can induce disturbances in the pituitary-adrenal gland system and thyroid gland in exposed rats and mice (OECD-SIDS, 2002a). Endocrine changes (e.g. adrenal glands, kidneys, liver, thymus) triggered by hexane were also reported in mammals (EHC, 1991). Nevertheless, in studies involving mouse, rats and humans described in ATSDR (1999) no histological changes were found in their endocrine tissues. Still, hexane was identified by the International Chemical Secretariat as a possible endocrine disruptor being included in SIN List 1.0 released by Chemical Secretariat (ChemSec, 2015). For isononanol and cyclohexylbenzene no information was available.

The paucity of knowledge about the endocrine and neurotoxic effects triggered in organisms exposed to HNS evinced the need for and importance of pursuing research. Data available is misleading and inconclusive and, in the case of aquatic organisms, the consequences of HNS contamination at this level are far from being known and understood.

5.5. Biomarkers for predicting HNS toxicity to marine organisms

Biomarkers are sensitive tools to determine the presence of contaminants and monitor ecosystem status (Vidal et al., 2001). Their use in integrated chemical and biological effects monitoring programmes of marine and coastal ecosystems within important maritime traffic routes could bring useful field information to contrast with laboratory based experiments. Ultimately, these programmes always provide baseline information, for different species and biomarkers. This baseline integrated characterisation is essential to evaluate effects of future accidental spills, contamination events, or natural disasters. Furthermore, the use of multiple biomarkers in controlled laboratory experiments with marine organisms (e.g. measurement of biochemical and cytotoxic responses) will provide EC data and anticipate changes at higher levels, such as in reproduction or behaviour (e.g. locomotion, feeding) triggered in the exposed organisms. This information will be valuable for the elaboration of more specific contingency plans.

The information previously reviewed provided the identification of several biomarkers with potential to determine the toxicity of HNS. First, potential target organs in vertebrates, or equivalent invertebrate tissues, identified for HNS biomarker studies are: i) the brain and stomach for acrylonitrile, rather than the liver; ii) the brain also for hexane, trichloroethylene and xylene; iii) the kidneys and liver for trichloroethylene, iv) lung and heart for xylene. Secondly, potentially sensitive biomarkers that could be used in the assessment of HNS toxicity to marine organisms are:

- the activity of enzymes involved on HNS metabolism and metabolite levels in urine, blood and plasma (Table 5) — as indication of HNS biotransformation;
- antioxidant defences and oxidative damage levels: content in ROS and endogenous antioxidant molecules; activities of antioxidant enzymes; extent of lipid peroxidation, protein oxidation and damage to nucleic acids, as measure of detrimental effects and protective mechanisms that have been triggered;
- neurotoxic parameters — considering that some HNS and their metabolites can be neurotoxicant, further research on these parameters would be quite relevant;
- blood chemistry and haematological parameters;
- cell viability and integrity;
- carcinogenicity signs such as abnormal cell proliferation and carcinogenic gene expression markers, peroxisome proliferation, occurrence of apoptosis or necrosis.

In addition, pharmacokinetic and pharmacodynamic models can also compose advantageous tools for predicting the toxic effects that may be induced in marine organisms explaining the complex relationship between the contaminant and the effects triggered (Tyler et al., 1993). As most work at this level has been focused mainly on mammals, a research line centred on aquatic organisms, and marine species in particular, must be followed in order to fulfil the current lack of knowledge and improve environmental risk assessment of these substances.

6. Final remarks

In view of the increasing maritime trade of HNS, estuarine and marine ecosystems are at high risk for HNS contamination. Hence, development of more effective legislation for the handling and transportation of hazardous substances and the establishment of environmental safety thresholds are crucial for the protection of these ecosystems. For that, more solid knowledge about the ecotoxicity of acrylonitrile, n-butyl acrylate, cyclohexylbenzene, hexane, isononanol, trichloroethylene and xylene is necessary. According to the results of this review, future studies should be oriented towards increasing the knowledge base in three lines, essential for the protection and estimation of legal compensations

for damage in affected areas. Evaluation of long-term effects of these HNSs in marine organisms, and investigation of their specific modes of action are two important lines. Here multi-biomarker batteries combined with other biological effects tools (e.g. histopathology, scope for growth, cellular energy allocation and feeding behaviour) will provide relevant insight. Using realistic HNS exposure scenarios will also help to improve protection of marine species. A third line, requiring priority attention, relates to the evaluation of ecological effects of these HNSs. There is evidence in the literature that at least some of these HNSs may affect growth and reproduction, with impact at the population level. Despite this, a huge lack of information from ecological studies was detected herein, which hampers accurate estimation of impact, establishment of financial compensations, and preparation of emergency response plans.

In terms of ecological protection, the investigation of non-destructive approaches to be used as early-warning tools of exposure/effect should also be a priority to minimise pain, distress and animals' sacrifice and guarantee the conservation of aquatic species, especially those endangered. Biological materials, such as blood, faeces, fur and skin biopsy specimens, have for example been used in studies involving vertebrates (Fossi and Marsili, 1997). The measurement of fluorescent model P-glycoprotein substrate rhodamine B in haemolymph, plasma and haemocytes (Žaja et al., 2006) and the clearance rate (Toro et al., 2003) has been applied to invertebrates. Procedures using non-destructive markers must be refined in order to accurately relate their degree of change to higher levels effects and to the concentrations of contaminants to which the organism are exposed. In the near future, this early diagnosis approach should be applied in ecotoxicological studies in detriment to more invasive ones.

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