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Seawater ecotoxicity of monoethanolamine, diethanolamine and triethanolamine

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ABSTRACT

Monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA) are compounds with potential acute, sub-chronic and chronic toxicity effects towards aquatic species. A literature review highlighted the existence of a gap in the knowledge on their toxicity with saltwater testing species. A battery of toxicity tests including the alga *Phaeodactylum tricornutum* Bohlin, the bivalve molluscs *Crassostrea gigas* (Thunberg) and *Mytilus galloprovincialis* (Lmk), and the crustacean *Artemia franciscana*, was considered to update and improve the existing ecotoxicological information. Data were provided as the Effective Concentration that induces a 50% effect in the observed population (EC50), Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC). EC50, LOEC and NOEC values were compared with a reviewed database containing the existing ecotoxicological data from saltwater organisms.

1. Introduction

Monoethanolamine (MEA) (2-aminoethanol, CAS 141-43-5), diethanolamine (DEA) (2,2′-iminodiethanol, CAS 111-42-2) and triethanolamine (TEA) (2,2′,2″-nitrilotriethanol, CAS 102-71-6) belong to the ethanolamine family and have a broad range of applications from industry to daily domestic use (*e.g.* cosmetics and personal care products) [1,2]. They are frequently applied in amine based processes for the removal of acid impurities from process gas streams and of CO_2 in industry [3,4]. MEA and DEA, as well as their mixtures, are also used for natural gas sweetening operations [4]. They might therefore be found in waste gas and wastewater, as well as their by-products due to their complete miscibility in water (20 °C) [5]. Indeed, on the basis of Mackay and Paterson fugacity model they tend to partition almost exclusively into the water compartment: 99.14% for MEA, 99.99% for DEA and 100.00% for TEA [5].

As a consequence of their wide range of uses and applications, their safety for human health and the environment must be carefully assessed.

A review by Davis and Carpenter [5] summarised their toxicological and ecotoxicological properties, indicating a gap in the knowledge about toxicity data with seawater species. Only limited and divergent information is actually available, mainly for some decomposer, producer and first consumer organisms [1,2,5,6]. The species have been short-listed as follows, indicating in brackets the compounds for which some data are available: *Vibrio fischeri* (MEA, DEA and TEA) [1,2], *Skeletonema costatum* (MEA and DEA) [4,5], *Chlorella vulgaris* (DEA) [1], *Artemia salina* (DEA and TEA) [5,7], *Crangon crangon* (MEA and TEA) [7] and *Asterias forbesi* (DEA) [5]. In particular, *A. salina* datum for DEA has been signalled as outlier by Pan Pesticide database [7] and the same may be suspected for TEA value.

Furthermore, the MEA, DEA and TEA potential for degradation was based on soil and freshwater [5], not on the marine environment, where it is recognised that the rate of degradation is generally slower than in the freshwater system [4], opening scenarios of concentration exposures having potential adverse effects. Indeed, a recent study [4] stated that MEA and DEA presented biodegradation values after 28 days incubation at 20 ± 1 °C in the dark lower than 60% as Theoretical Oxygen Demand (ThOD), which is considered the lower limit for a chemical to be released without having information on its potential ecotoxicity, while TEA has substantially shown not to degrade in seawater (<20% biodegradation as ThOD) according to the same experimental conditions [8].

Conflicting assessments of MEA, DEA and TEA (eco)toxicological implications have been reported. Davis and Carpenter [5] stated that these ethanolamines might be classified as "practically non toxic to slightly toxic" on the basis of *Daphnia magna* and *Ceriodaphnia dubia* acute tests. Conversely, Zurita et al. [1], ranking in accordance with 2001/59/EC directive guidelines [9], evidenced that DEA might be classified as "R52/53 Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment".

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Considering the PAN Pesticide database [7], MEA shows a moderate acute toxicity, DEA presents no available weight-of-the-evidence toxicity summary assessment and TEA is considered as not acutely toxic.

Marine species could be considered as a potential target of MEA, DEA and TEA that is present, not only in municipal wastewater, but also in oily wastewater, which is a major environmental problem especially in the coastal zone where chemical industries produce significant amounts of industrial wastewater [2,10]. Anyway, to the best of our knowledge there are no available data about environmental concentrations of MEA, DEA and TEA in discharges, surface water, soil or sediment.

A battery of model systems representing three trophic levels was selected, including growth inhibition (acute) of the alga *Phaeodactylum tricornutum* Bohlin, embryo larval development inhibition (sub-chronic) of the oyster *Crassostrea gigas* (Thunberg) and the mussel *Mytilus galloprovincialis* (Lmk), and immobilisation (acute) of the crustacean *Artemia franciscana*, in order to update and improve the existing ecotoxicological information.

The acute test with *P. tricornutum* is internationally recognised and standardised as ISO [11]. The sub-chronic test with *C. gigas* and *M. galloprovincialis* are worldwide well established for sediment, water column and wastewater assessment according to Whole Effluent Toxicity (WET) procedures since 1995 in the United States [12], and subsequently by ASTM [13], Rijkswaterstaat (RIKZ) [14], OSPAR [8] and Scottish Environmental Protection Agency (SEPA) [15] and most recent Italian regulation [16]. This study intentionally proposed both oyster and mussel toxicity data to compare their relative sensitivities and to show that it is possible to cover the laboratory activity with the same end-point (sub-chronic, embryo larval development) all the year around as well as to comply with cost-effectiveness rationale. In fact, mussels are available from the wild for toxicity testing only during the reproductive season (from October to April, Adriatic sea), while oysters may be available all the year around from specialised sea-farms, due to conditioning procedures, but at higher costs.

Nevertheless at international level the use of *Artemia* spp. in toxicity testing is subjected to a broad discussion with supporters [17,18] and detractors [19,20], *A. franciscana* acute immobilisation test was considered in this study because it is the only native crustacean bioassay recognised by the Italian Environmental Protection Agency [21] and Italian Water Act [22] for monitoring wastewater discharges to saltwater environments. Besides, the existing *A. salina* DEA and, probably, TEA toxicity data are outliers [7], so it is worth to obtain *A. franciscana* ones.

Moreover, it was decided not to include fish testing due to European recommendations about reducing vertebrate organisms toxicity testing (Directive 86/609/EEC).

2. Materials and methods

2.1. Toxicant exposure and analysis

A range of exposure concentrations of MEA (Baker, Deventer, Holland), DEA (Baker, Deventer, Holland) and TEA (Carlo Erba, Rodano, Italy) was firstly prepared in ultra-pure water and then diluted with various culture media according to the appropriate bioassay. MEA, DEA and TEA concentrations were determined by 761 Compact IC Ion chromatography (Metrohm AG, Herisau, Switzerland). Toxicity tests were performed in three replicate experiments using at least five geometrically scaled dilutions per each compound concentration. Starting solutions were analytically determined to be at 98.34 mg l^{-1} for MEA, at 498.54 mg l^{-1} for DEA and at 907.97 mg l^{-1} for TEA. Fresh dilutions were prepared just before each test run.

Salinity was checked with a hand refractometer, pH with an HI 9025 microprocessor-based pH meter (Hanna Instruments, Beverly, MA, USA) and dissolved oxygen with a WTW multiparametric device (Nova Analytics, Weilheim, Germany) in order to verify that the values were in accordance with each relative toxicity test protocol.

2.2. Toxicity tests

2.2.1. P. tricornutum

Growth inhibition of *P. tricornutum* was evaluated according to the ISO protocol [11] and certified mono-specific algal cultures were purchased from UGent (Belgium) (PT190608). The algal culture was kept at 20 ± 2 °C and 6000–10,000 lx, obtaining a cellular density of more than 10^6 cells ml^{-1}. The initial algal density in the test was obtained by dilution of algal culture and ranged between 2×10^3 cells ml^{-1} and 10^4 cells ml^{-1}. *P. tricornutum* was exposed to increasing concentrations of compounds for 72 ± 2 h at 20 ± 2 °C and 6000–10,000 lx, with a light/dark period of 16/8 h.

Negative and positive ($K_2Cr_2O_7$ as reference toxicant) controls were included in each experiment. Cellular density was evaluated using a Bürker counting chamber.

2.2.2. C. gigas and M. galloprovincialis

Embryo larval development with *C. gigas* and *M. galloprovincialis* was carried out according to ASTM [13] standard protocol modified for gamete pool as reported in Libralato et al. [23]. Conditioned oysters were purchased from the Guernsey Sea Farm Hatchery (Guernsey, UK), while mussels were caught from a sea farm in the northern Adriatic (Venice, Italy).

Good quality gametes from the best males and females, induced to spawn by thermal stimulation, were selected and filtered at 32 μm (sperm) and 100 μm (eggs) to remove impurities. A pool of eggs from at least three females (1000 ml) was fertilised by injecting 10 ml of sperm suspension; fertilisation was verified by microscopy. Egg density was determined by counting four sub-samples of known volume. Fertilised eggs, added to test solutions in order to obtain a density of 60–70 eggs ml^{-1}, were incubated for 24 h at 24 ± 1 °C for oysters and for 48 h at 18 ± 1 °C for mussels in 3 ml volume dilutions that had been pre-prepared in 3 ml 24 wells sterile polystyrene micro-plates with lids. At the end of the test, samples were fixed with buffered formalin and 100 larvae were counted, distinguishing between normal larvae and abnormalities. Negative and positive ($Cu(NO_3)_2$ as reference toxicant) controls were included in each experiment.

2.2.3. A. franciscana

Immobilisation of brine shrimp was assayed using APAT procedures [21]. *A. franciscana* certified cysts (AF/N2000) purchased from UGent (Belgium) were incubated (100 mg) in 12 ml of artificial seawater (Instant Ocean®, 35‰) at 25 ± 2 °C for 24 ± 2 h (1 h under artificial light, 3000–4000 lx, and the remainder in darkness) at pH 8.20. After incubation for 24 h, nauplii were collected with a Pasteur pipette and kept for an additional 24 h under the same conditions to reach the meta-nauplii stage. About 10 nauplii were transferred to each 3 ml well of polystyrene plates (24 wells with lids) containing the samples (2 ml of total volume). Negative and positive ($CuSO_4$ as reference toxicant) controls were included in each experiment. Twenty-four hours later, the number of survivors was counted and recorded.

2.3. Data analysis

Toxicity data were expressed as Effective Concentration that induces a 50% effect in the observed population (EC50) and its rel-

ative 95% confidence limits values, both based on the recorded Percentage of Effect (PE). The responses for each treatment (% of abnormalities) were corrected for effects in control tests by applying Abbott's formula [13]. The hypothesis test was conducted using Toxcalc software (v5.0.32) via Dunnett's method considering an arcsin $P^{1/2}$ transformation and the Trimmed Spearman Karber method for points estimation [13]. The Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) values were also calculated with the Dunnett program.

Moreover, the USEPA descriptive categories from Zucker [24] have been considered to rank toxicity values (<0.1 mg l^{-1}, very highly toxic; 0.1–1.0 mg l^{-1}, highly toxic; >1.0–10 mg l^{-1}, moderately toxic; >10–100 mg l^{-1}, slightly toxic; >100 mg l^{-1}, practically non-toxic).

3. Results and discussion

Ecotoxicological results are summarised in Table 1 as EC50, LOEC and NOEC values, while in Figs. 1–4 the entire dose–response curves for MEA, DEA and TEA are displayed for *P. tricornutum*, *C. gigas*, *M. galloprovincialis* and *A. franciscana*, in that order. From Figs. 1–4 presenting the *x*-axis log-scaled, it can be observed that the curves of MEA, DEA and TEA are always positioned from left to right for all testing species. This means that MEA is always more toxic than DEA, as well as TEA (EC50(MEA) < EC50(DEA) < EC50(TEA)).

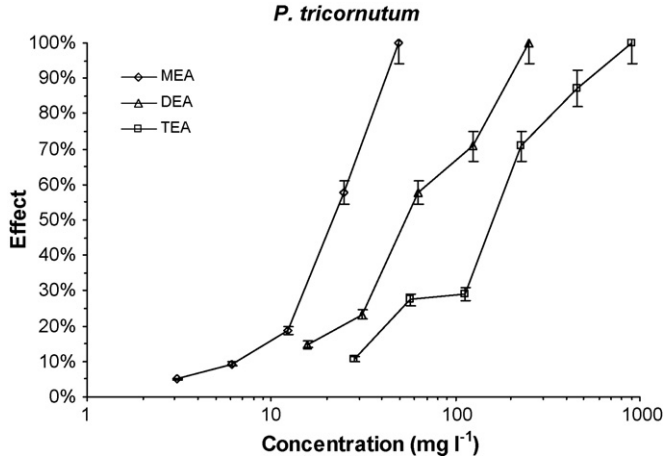

Fig. 1. Dose–effect relationship of *P. tricornutum* exposed to increasing concentrations of MEA, DEA and TEA.

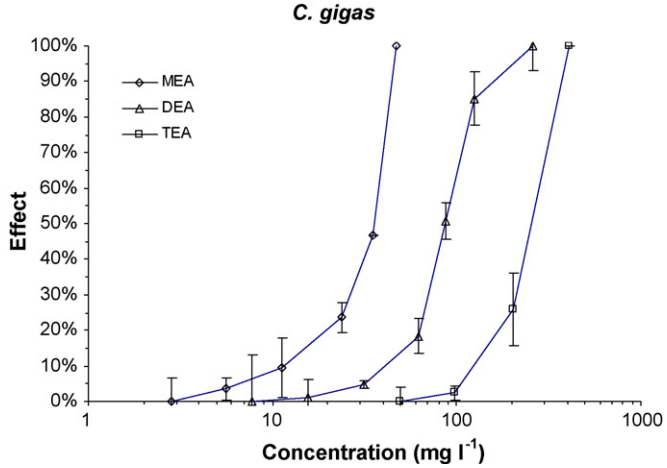

Fig. 2. Dose–effect relationship of *C. gigas* exposed to increasing concentrations of MEA, DEA and TEA.

Table 1
Toxicity data as EC50, LOEC and NOEC for the considered testing species both as mg l^{-1} and mM l^{-1}.

Test species	Unit	MEA			DEA			TEA		
		EC50	LOEC	NOEC	EC50	LOEC	NOEC	EC50	LOEC	NOEC
Alga										
P. tricornutum	mg l^{-1}	24.70 (17.90–31.50)	6	<6	86.96 (32.40–142)	16	<16	204 (105–303)	28	<28
	mM l^{-1}	0.40 (0.29–0.52)	0.10	<0.10	0.83 (0.31–1.35)	0.15	<0.15	1.37 (0.70–2.03)	0.19	<0.19
Molluscs										
C. gigas	mg l^{-1}	27.57 (26.37–28.82)	11.35	5.58	82.68 (79.25–86.27)	62.46	31.23	236 (229–244)	205	98
	mM l^{-1}	0.45 (0.43–0.47)	0.19	0.10	0.79 (0.75–0.82)	0.59	0.3	1.58 (1.53–1.64)	1.37	0.66
M. galloprovincialis	mg l^{-1}	18.17 (17.85–20.20)	0.09	<0.09	71.72 (65.24–78.85)	1.98	0.94	112 (97–131)	0.74	<0.74
	mM l^{-1}	0.29 (0.29–0.34)	0.001	<0.001	0.68 (0.62–0.75)	0.02	0.009	0.75 (0.65–0.88)	0.005	<0.005
Crustacean										
A. franciscana	mg l^{-1}	43.00 (35.98–51.38)	2.85	<2.85	378 (313–458)	124.92	62.46	577 (477–698)	150	100
	mM l^{-1}	0.70 (0.59–0.84)	0.05	<0.05	3.60 (2.98–4.36)	1.19	0.59	3.87 (3.20–4.68)	1	0.67

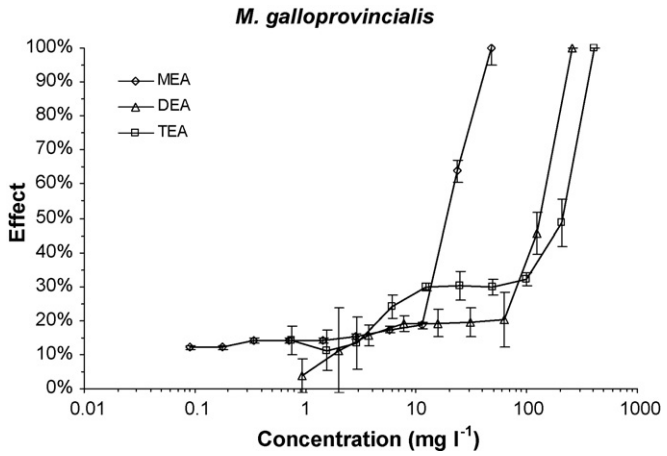

Fig. 3. Dose–effect relationship of *M. galloprovincialis* exposed to increasing concentrations of MEA, DEA and TEA.

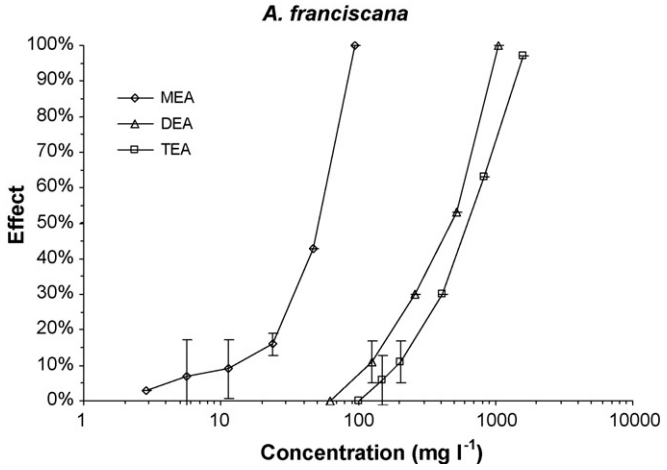

Fig. 4. Dose–effect relationship of *A. franciscana* exposed to increasing concentrations of MEA, DEA and TEA.

MEA EC50 values are characterised by the same order of magnitude, ranging from 18.17 mg l^{-1} with *M. galloprovincialis* to 43.00 mg l^{-1} with *A. franciscana*. DEA toxicity showed comparable values between the alga and the two molluscs, that is from 71.72 mg l^{-1} with the mussel and 82.68 mg l^{-1} with the oyster to 86.96 mg l^{-1} with the alga, while the brine shrimp evidenced a sensitivity more than one order of magnitude lower (378 mg l^{-1}). Regarding TEA EC50 s, the oyster and the alga presented similar values, 204 mg l^{-1} and 236 mg l^{-1}, the mussel displayed an EC50 that was about half of those (112 mg l^{-1}), whereas the EC50 of *A. franciscana* was almost double (577 mg l^{-1}).

Previous literature toxicity data about MEA, DEA and TEA are displayed in Table 2. The proposed database presents several gaps and contrasting information, especially for DEA, which has EC50s that diverge by one to two orders of magnitude.

The most sensitive species is *A. forbesi* with a DEA EC50 of 10 mg l^{-1}, conversely the least sensitive in absolute terms is *A. salina*. *A. salina* data for DEA and TEA appear as outliers compared to all other relative data in Table 2, as well as those with *A. franciscana* reported in Table 1, which are one order of magnitude lower for both compounds. Only Libralato et al. [2] provided full information for MEA, DEA and TEA with *V. fischeri* according to three exposure times (5, 15 and 30 min). In particular, DEA EC50 at 5 min exposure time with *V. fischeri* from Zurita et al. [1] with unknown confidence limit values is about half that from Libralato et al. [2]. Anyway, Libralato et al. [2] data seem to be in accordance with those presented in Table 1 and indicate a decreasing toxicity from MEA to DEA to TEA. In this specific case study, the sensitivity of *V. fischeri* was shown to be very similar to that of *P. tricornutum* and *C. gigas*.

As regards algae, *P. tricornutum* was shown to be about one order of magnitude more sensitive to DEA than *C. vulgaris* and *S. costatum*.

Applying the descriptive categories of Zucker [24] to toxicity responses, MEA can be classified as slightly toxic as well as DEA, except for *A. franciscana* for which it appeared to be practically non-toxic. Although TEA might be ranked as practically non toxic on the basis of all testing species outputs, it was shown not to degrade in seawater (<20% biodegradation as ThOD after 28 days incubation at 20 ± 1 °C in the dark) [4], opening scenarios about medium- and

Table 2
Review of existing literature toxicity data as EC50 for MEA, DEA and TEA according to various seawater testing species.

Test species	End-point	MEA mg l^{-1}	DEA mg l^{-1}	TEA mg l^{-1}	Reference
Bacterium					
V. fischeri 5 min	Bioluminescence inhibition	–	60	–	[1]
		26.37 (23.24–29.93)	122 (117–128)	547 (504–595)	[2]
V. fischeri 15 min		23.52 (19.51–28.36)	111 (107–116)	503 (476–536)	[2]
V. fischeri 30 min		21.50 (18.60–24.86)	95.51 (90.63–100.66)	425 (399–453)	[2]
Algae					
C. vulgaris 72 h	Growth inhibition	–	778	–	[1]
S. costatum 72 h		–	523[a]	–	[5]
S. costatum 72 h		100–200	200–400	–	[4]
Crustaceans					
A. salina 24 h	Immobilisation	-	-	5600[b]	[5]
A. salina 96 h		–	2800[b,c]	–	[5]
C. crangon		100	–	100	[7]
Echinoderm					
A. forbesi	Toxicity threshold for cell multiplication inhibition test	–	10[d]	–	[5]

–: value not available.
[a] Cowgill et al. [25].
[b] Price et al. [26].
[c] Considered as an outlier by Pan Pesticide [7].
[d] Bringmann and Kühn [27].

long-term exposures for marine species causing potential adverse effects. Considering the minimum recommended EC50 value concentration not to be exceeded of 10 mg l^{-1} for the protection of the marine phytoplankton stated by the Norwegian Pollution Control Authority within OSPAR [8], MEA, DEA and TEA EC50 results are above the acceptable value for *P. tricornutum*, confirming Eide-Haugmo et al. [4] toxicity data for MEA and DEA with *S. costatum*.

However, slightly acute toxicity may still pose potential significant chronic hazards (cancer, reproductive and developmental toxicity, endocrine disruption or genetic effects) or cause behavioural changes that might affect species survival. Moreover, due to the fact that MEA, DEA and TEA frequently occur in mixtures, interactive effects (additive, more than additive and less than additive) between them and with other chemicals cannot be excluded and should be further investigated.

4. Conclusions

In this work, the ecotoxicological assessment of MEA, DEA and TEA according to the considered test battery (alga, mussel, oyster and crustacean) updated and improved the existing database for saltwater species. It was demonstrated that MEA and DEA have slight acute toxicities and that the combination with their biodegradability might promote potential long-term toxicity effects towards aquatic organisms. In addition, even though TEA might be classified as not presenting acute toxicity, it could, like MEA and DEA, exert long-term chronic effects due to its low seawater biodegradability. Further research will be necessary on potential mixture effects and should be also aimed at understanding the potential toxicity of their by-products to marine life.

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