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Short communication

Toxicity evaluation of diethylene glycol and its combined effects with produced waters of off-shore gas platforms in the Adriatic Sea (Italy): Bioassays with marine/estuarine species

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ABSTRACT

Diethylene glycol (DEG) is commonly used to dehydrate natural gas in off-shore extraction plants and to prevent formation of gas hydrates. It may be released into the sea accidentally or in discharged produced waters (PWs). PWs samples from off-shore gas platforms in the Adriatic Sea (Italy) have been used in this study. The objectives of the study were: a) to evaluate the toxicity of DEG for marine organisms; b) to evaluate if a high DEG content in PWs may alter their toxicity; c) to verify whether the DEG threshold concentration established by the Italian legislation (3.5 g/l) for PWs discharged at sea is safe for marine environment. Ten different species (*Vibrio fischeri*, *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, *Brachionus plicatilis*, *Artemia franciscana*, *Tigropus fulvus*, *Mytilus galloprovincialis*, *Crassostrea gigas*, *Tapes philippinarum* and *Dicentrarchus labrax*) have been exposed to DEG; four of these species were also exposed to PWs in combination with DEG. The results showed that: a) DEG is not toxic at levels normally detected in Adriatic PWs; b) DEG in combination with PW showed mainly additive or synergistic effects; c) short-term bioassays showed that the DEG limit of 3.5 g/l could be acceptable.

1. Introduction

During off-shore extraction processes, natural gas is normally saturated with water vapour and thereby water molecules could combine with hydrocarbons to form crystalline-structured solid hydrates (Carroll, 2009). Glycols (triethylenic glycol, diethylene glycol and ethylene glycol) are the chemicals most often used in dehydration of natural gas (Sorensen et al., 2000). They are a group of organic compounds named aliphatic alcohols which are characterized by the presence of two hydroxy functional groups linked to methyl subunits (AIHA, 1985). In a typical glycol-based dehydration device, water vapour is removed from the gas stream in a glycol-absorber and the dried gas then leaves the absorber for further processing or transport (Katz and Lee, 1990). The water-laden glycols are usually regenerated (stripped of water) in a stripping column (Twijnstra, 1997). Because the efficiency of the regeneration processes occurring in the glycol-based dehydration devices is less than 100%, glycols could be introduced in the marine environment through the discharge of production waters (PWs) from off-shore extraction plants (Cappiello et al., 2007).

PW originates from water naturally present in geological formations (formation water) mixed with the seawater injected in the oil/gas field (process water) to maintain reservoir pressure. It is piped to the surface during the production process and may be discharged into the sea when the rejection is not possible, becoming the major effluent discharged during the hydrocarbons production phase (Patin, 1999). Before discharge, PWs are treated directly on platform to reduce oil and suspended solids content but, in spite of this treatment, PWs still include many inorganic compounds (i.e. trace metals), volatile aromatic compounds (benzene, toluene, ethylbenzene, xylenes - BTEX), semi-volatile substances (i.e. naphthalene, phenanthrene, dibenzothiophene - NPD), phenols, organic acids and additives (e.g. glycols) (Brendehaugh et al., 1992; Neff, 2002). These compounds are present in variable amounts depending on the geological characteristics of the reservoir, the type of hydrocarbons (gas or oil), the degree of exploitation of the reservoir, and the efficiency of the treatment adopted (Utvik, 1999; Wills, 2000; OSPAR, 2004). Since PW composition is complex and very variable, toxicity can be different depending on its chemical characteristics (Higashi et al.,

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1992; Neff, 2002; Holdway, 2002). Discharge of PWs into the sea may cause impacts on the biota but usually adverse effects occur only within the mixing zone around the production platforms (Burns et al., 1999; Cianelli et al., 2008). Chemical characteristics, potential effects and modelling of PW dispersion have been investigated by several studies in areas characterized by the presence of off-shore platforms discharging PW into the sea (e.g. North Sea, Gulf of Mexico, Mediterranean Sea, coastal areas of Australia and of Southern California) (Cianelli et al., 2011 and references therein). Irrespective of the variations in their chemical composition, PWs have a relatively low toxicity (Holdway, 2002; Neff, 2002; OGP, 2005). Negligible or non-toxic effects have also been observed on marine organisms in the Adriatic Sea (Manfra et al., 2007; Cianelli et al., 2008). However, very few data are available on the potential impact of chemicals on PW toxicity (Henderson et al., 1999 on 11 chemicals including biocides, corrosion inhibitors and demulsifiers; Beyer et al., 2001) and very limited data are related to the toxicity of diethylene glycol (DEG) (Kent et al., 1999; Gorbi et al., 2009), that is in Italy the additive used for dehydration of natural gas and the most used additive in off-shore gas platforms.

DEG is a relatively non volatile compound, due to its low vapour pressure, and is water-soluble. The octanol-water partitioning coefficient is very low and hence bioaccumulation is not expected to be significant, while hydrolysis may be an important fate process for DEG in water (Kent et al., 1999). A maximum allowable concentration of DEG in PWs, equal to 3.5 g/l, has been established only in Italy (as required by Authorization Decrees of the Ministry of Environment), on the basis of few experimental data available on marine aquatic toxicity on *Cyprinodon variegatus*, *Skeletonema costatum*, *Mysidopsis bahia* and *Artemia salina* (Kent et al., 1999), that suggest a relatively low DEG toxicity. Previous studies (Cappiello et al., 2007; Cianelli et al., 2008) and 2001–2010 data on chemical characterization of PWs in the Adriatic Sea (data from authorization requests to the Ministry of the Environment for PW discharge at sea, unpublished) showed that DEG concentration in PWs is generally lower than the maximum allowable concentration and ranges from <0.5 mg/l to 13 mg/l. Furthermore, seawater concentrations of DEG after discharge at sea are further reduced by 5 folds to 1 order of magnitude due to dilution processes (Cianelli et al., 2008).

However, because of its high solubility in water and the presence of ethylene groups, DEG may act as a co-solvent in PW (Sorensen et al., 2000). A co-solvent has effects on the solubility and sorption (hence, on transport) of organic chemicals in an aqueous solution (Rao et al., 1991). Sorption of organic solutes, especially hydrophobic organic chemicals, is inversely related to solubility. Thus, an increase in solubility resulting from the addition of a co-solvent leads to a proportional decrease in sorption. DEG might facilitate the desorption on PW particulate phase of some organic compounds, including most aromatic compounds as BTEX and NPD. For these reasons, it is relevant to study DEG in combination with PW in order to assess the possible effects on PW's toxicity of an increased solubility of compounds with high toxicological interest. At present there are no data on the contribution of DEG to PW toxicity except for a single study on molecular/cellular effects on sea bass of DEG in combination with PW (Gorbi et al., 2009), that showed slight but significant synergic effects of this mixture.

The objectives of the present work were: i) to investigate the toxicity of DEG alone, using a test battery of marine/brackish organisms; ii) to evaluate if the presence of DEG in PWs may alter their toxicity; and iii) to establish a safe threshold concentration for DEG in combination with PWs. A combination of bioassays and biomarker methods, coupled with chemical analysis, was used for this purpose. Biomarker results and the results of the chemical analyses and toxicity tests on PWs have been published by Gorbi et al. (2009) and Manfra et al. (2010), respectively, while bioassay results on DEG and on the mixture DEG + PWs are reported in this paper. Two sets of toxicity tests have been carried out. In the first set, a battery of ten species was exposed to different DEG concentrations. The test species included bacteria (*Vibrio fischeri*), algae (*Phaeodactylum tricornutum* and *Dunaliella tertiolecta*) rotifers (*Brachionus plicatilis*), crustaceans (*Artemia franciscana* and *Tigropus fulvus*) molluscs (*Mytilus galloprovincialis*, *Crassostrea gigas*, *Tapes philippinarum*) and fish (*Dicentrarchus labrax*). In the second set of toxicity tests, one species for each trophic level (decomposers, primary producers, consumers and predators) was exposed to PWs in combination with DEG in order to assess possible synergistic effects and to verify whether the current threshold value of DEG in discharged PWs (3.5 g/l) is a safe concentration for the marine environment.

2. Materials and methods

2.1. Sampling

Samples of PWs were collected from three off-shore gas platforms located at about 20 km from the Adriatic coast (one near Pescara and two near Rimini, Italy). One sample for each platform has been taken: PW1 was collected in October 2005, PW2 and PW3 in June 2006. All PWs were sampled by high density polyethylene bottles from a tap located on the platform, downstream of the treatment plant, and then immediately filtered (Millipore™, 0.45 μm cellulose acetate membranes) and stored at 4 °C. Data on physico-chemical characteristics and on concentrations of chemical compounds in the three PWs have been presented in Manfra et al. (2010) and are summarized in Table 1. The bioassays were carried out within 72 h.

2.2. Experimental design

Two sets of toxicity tests have been carried out and each test was repeated independently three times. The toxicity tests were

Table 1
Physico-chemical parameters and concentrations of chemicals in the three production waters (PW) used in the study (ICRAM, 2006; Gorbi et al., 2009; Manfra et al., 2010).

Parameters	Unit	PW1	PW2	PW3
Salinity	PSU	34	37	37
pH		7	7	8
Conductivity	mS/cm^2	51	56	55
ORP	mV	−100	−105	−70
Dissolved Oxygen	%	93	96	86
Ba	mg/l	10.527	3.360	0.110
Cr	mg/l	<0.01	<0.01	<0.01
Cu	mg/l	0.02	<0.01	<0.01
Mn	mg/l	0.42	0.35	0.050
Ni	mg/l	0.02	<0.01	<0.01
Pb	mg/l	<0.01	<0.01	<0.01
Zn	mg/l	0.02	1.47	0.60
Cd	mg/l	<0.0005	<0.0005	<0.0005
Fe	mg/l	25.48	2.69	1.232
As	mg/l	<0.01	<0.01	0.10
Hg	mg/l	<0.0005	<0.0005	<0.0005
Benzene	mg/l	0.256	0.0104	0.0204
Toluene	mg/l	0.0506	0.0141	0.0121
Ethylbenzene	mg/l	0.1152	0.0077	0.0138
Xilenes (o,m,p-xylene)	mg/l	0.860	0.0148	0.0202
∑BTEX	mg/l	1.2818	0.047	0.0665
DEG	mg/l	2.4	9.6	13.0
∑Aliphatic hydrocarbons C7–C20	mg/l	0.034	0.0098	0.015
∑Polycyclic aromatic hydrocarbons	mg/l	0.150	0.100	0.126
∑Chlorophenols	mg/l	<0.002	<0.002	<0.002
∑Nitrophenols	mg/l	<0.002	<0.002	<0.002
∑Alkylphenols	mg/l	0.078	0.041	0.055