Science of the Total Environment 472 (2014) 302–308

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Toxicity of spill-treating agents and oil to sea urchin embryos

Diego Rial *, José A. Vázquez, Miguel A. Murado

Grupo de Reciclado y Valorización de Residuos (REVAL), Instituto de Investigaciones Marinas (IIM-CSIC), C/Eduardo Cabello, 6, Vigo, Pontevedra E-36208, Spain

HIGHLIGHTS

- The toxicity of the oil and STA WAFs to sea urchin embryos was assessed.
- The joint toxicity of binary mixtures was accurately described by CA and IA models.
- No synergistic toxicity was found for the mixtures of oil and STA WAFs.
- The dispersant and the dispersed oil were more toxic than the oil WAF.
- Finasol OSR51 significantly contributes to the CEWAF toxicity.

ARTICLE INFO

Article history:
Received 8 September 2013
Received in revised form 24 October 2013
Accepted 5 November 2013
Available online 30 November 2013

Keywords:
Crude oil
Dispersant
Joint toxicity
Sea urchin
Concentration Addition
Independent Action

ABSTRACT

The aim of this study was to assess the joint toxicity of a Maya crude oil and four spill-treating agents (STAs) (CytoSol, Finasol OSR51, Agma OSD569 and OD4000). The acute toxicity of the binary mixtures of the water accommodated fractions (WAFs) obtained independently for the oil and each STA was assessed. The toxicity of the chemically enhanced WAF (CEWAF) of oil and Finasol OSR51 at several dispersant to oil ratios (1:2, 1:10 and 1:100) was also evaluated. The toxicity (EC_{50}) obtained for the WAFs of the STAs was: CytoSol (15.1 mL/L) < Agma OSD 569 (9.8 mL/L) < OD4000 (2.6 mL/L) < and Finasol OSR 51 (1.8 mL/L). An accurate description of the toxicity of binary mixtures was obtained by the following models: Concentration Addition (Agma OSD569 and CytoSol), Independent Action (Finasol OSR51) and extended Concentration Addition model to describe antagonistic effects (OD4000). The CEWAFs showed an intermediate position between the more moderate effects of the WAF of oil and the higher toxicity of the WAFs of Finasol OSR51, which suggested the high sensitivity of the sea urchin embryo toward the dispersant.

1. Introduction

Spill treating agents are chemicals developed to assist in cleaning up or removing oil (Fingas, 2011). Dispersants are expected to promote the formation of small droplets of oil into the water column and shoreline washing agents are applied to the shorelines to release the oil (Fingas, 2011). In both cases, the spill-treating agent (STA) to oil ratio determines the effectiveness of the technique. A dispersant to oil ratio (DOR) of 1:20 is commonly considered optimal, although a ratio of 1:10 can be required for emulsified and viscous heavy oils (NRC, 2005). Ratios for surface washing agents are usually higher (1:5–1:2.5) (USEPA, 1993). Since the thickness of the oil layer varies, there will inevitably be areas with over-dosing and under-dosing in any dispersant or surface washing agent spraying operation (EMSA, 2010).

The action of an STA is related to a physical phenomenon resulting in an increased exposure to hydrocarbons in the water column. Toxicity of dispersed oil can be a result of: 1) dissolved materials; 2) particulate phase; and 3) enhanced uptake (Singer et al., 1998). Complications related to the testing of oil and STAs have led the Chemical Response to Oil Spills Environmental Research Forum (CROSERF) to propose standard conditions for assessing oil and dispersants in order to improve the comparability of the results (Singer et al., 2000). According to current knowledge, the toxicity of dissolved hydrocarbons has additive effects although there are doubts as to whether synergies exist between dissolved oil and chemically dispersed oil droplets (NRC, 2005). To characterise if the interaction among petroleum and a STA is synergistic, it would be necessary to consider the joint toxicity according to the reference hypotheses: Independent Action (IA) or Concentration Addition (CA).

Early life stages of sea urchin have been used in ecotoxicology due to sensitivity and low cost. Embryo toxicity tests using sea urchin have also been a pioneering tool for assessing the toxicity of oil (Allen, 1971; Falk-Petersen, 1979; Falk-Petersen and Lonning, 1984) and dispersants

* Corresponding author at: Grupo de Reciclado y Valorización de Materiales Residuales (REVAL), IIM-CSIC, Eduardo Cabello, 6, E-36208 Vigo, Spain. Tel.: +34 986231930; fax: +34 986292762.
E-mail address: diegorialconde@iim.csic.es (D. Rial).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.030

(Falk-Petersen et al., 1979; Kobayashi, 1981; Lonning and Falk-Petersen, 1979). Recently, Beiras et al. (2012) have proposed that the measure of larval growth as length allows a more gradual and objective quantification than abnormalities and facilitates its use as a standard test.

The present work aims to achieve a better understanding of the mechanisms of toxicity of oil and spill-treating agents. To study the existence of independent, additive, synergistic or antagonistic effects among Maya crude oil and four STAs, individual water accommodated fractions (WAFs) of both and binary mixtures were performed and toxicity was assessed by sea urchin embryo-larval test. The effect of different dispersant to oil ratios in the toxicity of the dispersed oil and the toxic contribution of dispersant were also evaluated.

2. Material and methods

2.1. Spill-treating agents and oil

Four STAs were selected in a previous report (Murado et al., 2008) as a result of their ability to remove fuel adhering to granite rock: the surface washing agent CytoSol (CytoCulture International, Inc., Point Richmond, California, USA); and the dispersants Finasol® OSR 51 (Total Special Fluids, Paris, France), Agma OSD 569 (Agma PLC, Northumberland, United Kingdom) and OD4000 (Innospec Ltd, Cheshire, United Kingdom). The products were kindly provided by the manufacturers or trade representatives. A Maya crude oil was provided by Repsol YPF S.A. (A Coruña, Spain).

2.2. Preparation of water accommodated fractions of oil and spill-treating agents

2.2.1. Binary mixtures of water accommodated fractions of oil and the STAs

In the first experiment, water accommodated fractions (WAFs) were obtained by adding 50 mL of Maya crude oil or the STA to 950 mL of 0.22 μm filtered sea water (FSW) and kept in an orbital shaker for 48 h (150 rpm/20 °C/darkness). The aqueous phase was separated and binary mixtures of WAFs and dilutions with FSW were performed for toxicity testing (Table 1).

2.2.2. Water accommodated fractions obtained with different ratios of Finasol OSR51 to oil, dispersant loadings and single oil loading

In the second experiment, chemically enhanced water-accommodated fractions (CEWAFs) of Maya crude and the dispersant Finasol OSR51 were obtained with the following DOR (dispersant:oil): 1:2 (25 mL/L:50 mL/L), 1:10 (5 mL/L:50 mL/L), and 1:100 (0.5 mL/L:50 mL/L) in a 2-L flask with a Teflon cap (Table 2). WAFs of oil (50 mL/L) and Finasol OSR51 (25, 5 and 0.5 mL/L) were also prepared in the amounts mentioned above (Table 2). The total volume of oil, dispersant, and water was 1 L in all cases. The mixture was kept in the dark and shaken (150 rpm) for 46 h at 20 °C and allowed to settle for 2 h. The water phase was syphoned and diluted with FSW for toxicity testing (Table 2).

Table 1
Dilutions of the WAFs of Maya crude oil and the spill-treating agents specified in the text. Concentrations used for the binary mixtures of the WAFs are underlined.

Experiment	WAF	Concentration (mL/L)
Oil–CytoSol	Oil	0, 50, 100, 200, 400, 500, 600, 700, 800, 960
	CytoSol	0, 1, 2, 5, 10, 20, 30, 40, 50, 100
Oil–Agma OSD569	Oil	0, 50, 100, 200, 400, 500, 600, 700, 800, 960
	Agma OSD569	0, 1, 2, 5, 10, 15, 25, 40, 100, 200
Oil–Finasol OSR51	Oil	0, 50, 100, 200, 400, 500, 600, 700, 800, 980
	Finasol OSR51	0, 0.1, 0.2, 0.5, 1, 1.5, 2, 3, 10, 20
Oil–OD4000	Oil	0, 100, 200, 400, 600, 800, 980
	OD4000	0, 0.2, 0.5, 1, 3, 7, 15

Table 2
Maya crude oil and Finasol OSR 51 loads, dispersant to oil ratios (DOR) and dilutions of the WAFs and CEWAFs used in this experiment.

Treatment*	WAF/CEWAF	Dispersant load (mL/L)	Oil load (mL/L)	DOR
A	WAF Oil	–	50	–
B	CEWAF	25	50	1:2
C	CEWAF	5	50	1:10
D	CEWAF	0.5	50	1:100
E	WAF dispersant	25	–	–
F	WAF dispersant	5	–	–
G	WAF dispersant	0.5	–	–

* The tested dilutions were 0, 2, 5, 10, 20, 50, 100, 200, 500 and 1000 mL/L in all treatments.

2.3. Chemical analyses

Total petroleum hydrocarbons (TPH) were determined by gas chromatography using a flame ionisation detector (FID) according to ISO 9377-2:2000 (ISO, 2001) and anionic surfactants as methylene blue active substances (MBAS) in accordance with standard 5540C (APHA et al., 1998) in the experiment of the joint accommodation of oil and Finasol OSR51. The WAFs were extracted three times with 50 mL of dichloromethane (DCM) in a separatory funnel; organic extracts were dried over anhydrous Na_2SO_4 and concentrated by vacuum evaporation.

Gas chromatography was performed using an Agilent GC 7890A (Agilent Technologies, Palo Alto, CA, USA) with autosampler 7693 and a flame ionisation detector (FID). A volume of 1 μL of each sample was injected in splitless mode (325 °C) in a G3510A Multimode Inlet with a septum purge flow of 3 mL/min. Separation was carried out on a HP-5MS capillary column (60 m × 25 mm, 0.25 μm film thickness) from Agilent (Agilent J&W, USA). Helium (ALPHAGAZ™ 2 B50 purity) was employed as a carrier gas at a constant linear average velocity of 18.84 cm s^{-1}. The GC oven temperature was programmed from 40 °C (held 1 min) to 325 °C at 6 °C min^{-1} holding the final temperature for 20 min (total analysis time: 68.5 min). The detector was operated at 325 °C.

The total chromatogram area corresponding to retention times from 12 to 50 min (n-Nonane and n-Triacontane) was quantified for a range of concentrations of Maya crude oil of 0.5–20 mg/mL after subtracting the area of a blank of hexane. In those samples containing oil and dispersant, the peaks corresponding to Finasol OSR51 were subtracted from the total area to quantify only the area corresponding to hydrocarbons.

A calibration curve with sodium dodecylbenzenesulphonate (CAS 25155-30-0) in the range 0.2–2 mg/L was prepared to determine the content of MBAS. The aqueous phase was diluted with distilled water prior to the determination of the concentration of anionic surfactants (0.5–4 mL sample, total volume 100 mL). According to the standard 5540C (APHA et al., 1998) a few drops of H_2O_2 were added to 100 mL of the diluted sample along with some drops of phenolphthalein, dropwise addition of NaOH 1N until there was a shift to basic pH and H_2SO_4 1N until a noticeable change to a transparent colour. 25 mL of methylene blue reagent was added and the sample was extracted three times with 10 mL of chloroform. A backwash of the chloroform was performed with 50 mL of wash solution. The chloroform was filtered through a funnel containing a plug of glass wool before measuring the absorbance at 652 nm.

2.4. Sea urchin embryo test

The sea urchin embryo test was performed in accordance with the method of Saco-Álvarez et al. (2010). Gametes of *Paracentrotus lividus* were obtained by dissection and maturity (ovum sphericity and sperm mobility) checked with a microscope. The ova were transferred to a 100 mL graduated cylinder containing sea water, a few drops of sperm taken from the male gonad were added using a Pasteur pipette, and the mixture was shaken gently to facilitate fertilisation. The