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Toxicity of binary mixtures of oil fractions to sea urchin embryos

Diego Rial[a,*], José A. Vázquez[a], Araceli Menduiña[a], Ana M. García[b], M. Pilar González[a], Jesús Mirón[a], Miguel A. Murado[a]

[a] Grupo de Reciclado y Valoración de Residuos (REVAL), Instituto de Investigaciones Marinas (IIM-CSIC), C/ Eduardo Cabello, 6. Vigo, Pontevedra, E-36208, Spain
[b] Servicio de Instrumentación y Calidad (SICIM), Instituto de Investigaciones Marinas (IIM-CSIC), C/ Eduardo Cabello, 6. Vigo, Pontevedra, E-36208, Spain

HIGHLIGHTS

- Fractionation of a Maya crude was performed into aliphatics, aromatics and polars.
- Toxicity of binary mixtures was assessed using a sea urchin embryo-larval test.
- Descriptive ability of Concentration Addition and Independent Action was compared.
- Concentration Addition was the best model to explain joint toxicity of fractions.

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ABSTRACT

The assumption of additive toxicity for oil compounds is related to a narcotic mode of action. However, the joint toxicity of oil fractions has not been fully investigated. A fractionation of Maya crude oil into aliphatics, aromatics and polars was performed, fractions were dissolved in dimethyl sulfoxide (DMSO) and subsequently toxicity of single fractions and binary mixtures was assessed using the sea urchin embryo test. The descriptive ability of Concentration Addition (CA), Independent Action (IA) and modifications of both models for describing the joint toxicity of mixtures has also been evaluated. The hydrocarbon content extractable with dichloromethane of the fractions dissolved in DMSO was: $12.0 \pm 1.8\ mg\ mL^{-1}$, $39.0 \pm 0.5\ mg\ mL^{-1}$ and $20.5 \pm 2.5\ mg\ mL^{-1}$ for aliphatics, aromatics and polars, respectively. The toxicity of the extracts in DMSO of the fractions as EC_{50} ($\mu L\ L^{-1}$) was: aliphatics (165.8–242.3) < polars (87.1–115.7) < aromatics (20.5–34.6). The goodness of fit of the CA model was in most binary mixtures (aliphatics–aromatics, aromatics–polars) greater than the IA (aliphatics–polars) according to the Akaike Information Criterion, so CA was considered a better option than IA to explain the joint toxicity of oil fractions. In addition, synergistic or antagonistic effects were not observed.

1. Introduction

Oil is a complex mixture of hydrocarbons whose composition after a spill depends on its geographical origin, refining process, along with the weathering and mixing in the environment [1]. The toxicity assessment of a mixture of compounds relies on exposure data, the effects of each compound data and the complexity of the mixture [2]. The assessment and interpretation of oil toxicity is a complex task addressed by different approaches in the past.

The hydrocarbon block method (HBM) [3] is an approach to environmental risk assessment aimed to petroleum substances. It is based on grouping compounds with similar physicochemical properties, assuming a narcotic mode of action for hydrocarbons and additive toxicity between blocks. These assumptions have been validated by numerous acute toxicity studies [4–7]. The predictive character of the HBM depends on several factors. In the first place, the resolution of the chromatographic method and assignation of mass fractions for each block. Secondly, that toxicity of the compounds could be known or, at least, estimated by quantitative structure-activity relationships. And finally, the realism of the estimations of its distribution in the environmental compartments (air, water, oil). The LC_{50} of a narcotic compound is estimated by the target lipid model (TLM) using the values of critical body burden for a given species, the octanol-water partition coefficient and a chemical class correction [8]. It is not possible to resolve chromatographically all compounds that belong to a certain type of oil, nor know the properties of each compound, its mode of action or other factors involved in toxicity (secondary mode of action, uptake kinetics, transportation, metabolism, compartmentation and excretion) [9]. This highlights the practical usefulness of

* Corresponding author. Tel.: +34 986231930; fax: +34 986292762.
E-mail addresses: diegorialconde@iim.csic.es, diegorialconde@uvigo.es (D. Rial).

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the HBM for predicting acute or chronic lethality given the current state of science. However, the high toxic potency of PAHs [8], their phototoxicity [10], and specific modes of action [11] raises doubts about the assumption of additivity.

The content of total petroleum hydrocarbons (TPH) in a sediment or water sample does not provide information about its composition nor serve to correlate the observed toxicity with a certain group of compounds. Due to this, whole mixture approaches combining chemical fractionation of oil with toxicity tests have been used in order to identify the most toxic components [6,12]. The characterization of oil properties is commonly performed by the content of classes of compounds – saturates, aromatics, resins and asphaltenes – or fractions composition corresponding to a range of equivalent carbon number (*e.g.* >EC10–EC16) [13,14]. The toxicity of a fraction sums up the additive, synergistic or antagonistic effects of all sample compounds including the unresolved complex mixture (UCM); though the predictive capability of such an approach highly depends on the similarity of the tested fractions [15,16].

The toxicity of a chemical mixture is usually assessed by two reference models: concentration addition (CA) and independent action (IA). Reliability in one of the models is expected to depend on the characteristics of the compounds. This is, CA model would be a better option for compounds with a common mode or mechanism of action, whereas IA model would be a better choice for dissimilarly acting compounds [17].

Sea urchins are economically and ecologically important species whose embryonic development is highly sensitive to oil [18]. The measure of length for the embryo-larval test instead of abnormalities has allowed a considerable standardization and a high consistency of the results [19]. This test, among other purposes, has been successfully used for testing the toxicity of: (a) a mixture of polycyclic aromatic hydrocarbons [10], (b) the water accommodated fraction following weathering [20], or (c) water and sediment samples taken after an oil spill [21].

The aim of this work was to gain a better understanding of the acute toxicity of the mixtures of coarse hydrocarbon fractions. For this purpose, aliphatics, aromatics and polars were isolated by column chromatography from a Maya crude oil. Extractions with dimethyl sulfoxide of each fraction and mixtures of extracts were performed and the toxicity of the binary mixtures assessed by a sea urchin embryo-larval test. The predictive ability of the CA and IA models was compared to describe the toxicity of the mixtures of fractions (aliphatics–aromatics, aliphatics–polars and aromatics–polars).

2. Materials and methods

2.1. Coarse fractionation

Maya crude oil was provided by Repsol YPF S.A. (A Coruña, Spain). The asphaltenes were precipitated in n-pentane (20 g oil: 200 mL pentane) by centrifugation at 931 × *g* for 10 min. Asphaltenes were washed 6 times by resuspension in an ultrasonic bath (20 min) and centrifugation (931 × *g* for 10 min) to minimize the adsorbed hydrocarbons. The deasphalted fraction was concentrated on a rotary evaporator at reduced pressure and 25 °C.

20 g of deasphalted fraction was added to a glass column (75 cm × 4 cm) packed with activated silica gel (70–230 mesh; 450 g) (Sigma-Aldrich, Inc. St. Louis, USA). The column was eluted with 3 L of pentane, 2 L of dichloromethane: pentane (85:15, v:v) and 2 L of dichloromethane: methanol (1:1, v:v) to obtain saturated, aromatic and polars, respectively. Solvents were partially removed in the rotavap at reduced pressure and 25 °C. A slight amount of each fraction was dissolved in hexane for chemical analyses.

2.2. Extraction in dimethyl sulfoxide and test mixtures

The solvent of the aliphatic, aromatic and polar fractions (~1 g) was evaporated by a gentle nitrogen stream. Dimethyl sulfoxide (DMSO) was selected as a carrier for oil because of low toxicity (8 mL L^{-1} NOEC/48 h for *Paracentrotus lividus*) [22] and ability to dissolve the potential biological active compounds [23]. DMSO was added at a 9:1 ratio (DMSO: oil fraction, w:w), extracted by orbital shaking (150 rpm) for 24 h at 50 °C and the extract of each fraction in DMSO removed with a microsyringe.

The range of concentrations to be tested was determined in a preliminary experiment (data not shown). The pure extracts were diluted with DMSO by microsyringe in vials with fused-in 300 μL inserts to obtain the single doses and binary mixtures of the fractions.

2.3. Chemical analyses

1 mL of the DMSO extract of each fraction was added to 1 L of 0.22 μm filtered sea water (FSW) and hydrocarbons were extracted per triplicate with 50 mL of dichloromethane (DCM). The organic extracts were dried over anhydrous Na_2SO_4, concentrated by vacuum evaporation and diluted to 10 mL with DCM. Hydrocarbon content of the fractions was determined per triplicate in samples of 3 mL by gravimetric quantification. The remaining volume of the sample was concentrated 10 times for chromatographic analysis (only for aliphatics and aromatics).

The chemical analyses were performed using an Agilent GC-MSD system (Autosampler 7693, GC 7890A, MSD 5975C) operated by MSD Productivity ChemStation Software (Rev. E.02.00 SP2). 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 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). Two detectors were used: a selective mass detector, 5975C inert MSD with Triple-Axis Detector in mode scan 40–450, and a flame ionization detector (FID) at 325 °C. A volume of 1 μL of each fraction was injected in mode splitless (325 °C) with a septum purge flow of 3 mL min^{-1}.

A search of the quantification and confirmation ions of aliphatics, mono- and polycyclic aromatic hydrocarbons was performed according to the CEN Methodology [24] and it was concluded that the fractionation obtained was correct.

2.4. Sea urchin embryo test

The sea urchin embryo test was performed in accordance with the method of Saco-Álvarez et al. [25]. Gametes of *P. 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 through a Pasteur pipette, and the mixture shaken gently to facilitate fertilization. The fertilization rate was determined in quadruplicate in samples of 100 individuals, as the proportion of eggs with a fertilization membrane. Within 30 min, the fertilized eggs were transferred to glass vials with 10 mL of FSW. The medium was dosed with 1 mL L^{-1} of the extract in DMSO of the fraction or mixture of fractions to be tested and the vials shaken in a vortex mixer (Table 1). Each vial received 40 eggs mL^{-1} and each dose was performed in quadruplicate (solvent and FSW controls in quintuplicate).

Vials were sealed, the eggs were incubated in the dark at 20 °C for 48 h, and the larvae fixed by adding a few drops of 40% formalin. The maximum length of 35 individuals was measured directly in