

Acute Toxicity of a Shoreline Cleaner, CytoSol, Mixed With Oil and Ecological Risk Assessment of its Use on the Galician Coast

Diego Rial · Ricardo Beiras · José A. Vázquez · Miguel A. Murado

Received: 29 October 2009 / Accepted: 16 February 2010 / Published online: 10 March 2010
© Springer Science+Business Media, LLC 2010

Abstract The application of embryo–larval bioassay with the purple sea urchin *Paracentrotus lividus* and the mussel *Mytilus galloprovincialis* at 48 hours, and with neonates of the mysid *Siriella armata* at 96 hours, was used to evaluate the acute toxicities of the following preparations: (1) the shoreline cleaning agent CytoSol; (2) the water-accommodated fraction of CytoSol plus a light crude oil; and (3) the runoff from a pilot-scale treatment with CytoSol of a rocky coastal substrate impregnated with residues from the *Prestige* oil spill (which occurred on November 19, 2002). The mussel was the most sensitive organism to CytoSol and runoff effects ($EC_{50} = 8.0 \mu\text{L/L}$ and 64.3 mL/L , respectively), and the mysid was the least sensitive to the runoff ($EC_{50} > 200 \text{ mL/L}$). The predicted no-effect environmental concentration (PNEC) was calculated from the no observed–effect concentration of the species most sensitive to the runoff. The predicted environmental concentration (PEC) was estimated from a simple and reasonable dilution model, and the PEC/PNEC ratio was calculated according to the area treated and the values of the variables considered in the model. Implications for the management of the treatment operations are discussed.

On November 19, 2002, the oil tanker *Prestige* sank 209 km off Cape Fisterra (NW Iberian Peninsula), spilling

part of its load of 65,000 metric tons of no. 6 fuel oil and causing damage to >800 km of the Atlantic coast (Rousseau 2003). After the accident, the affected coast was cleaned using a variety of methods: installation of barriers and manual or mechanical removal, high-pressure water washing, and bioremediation (CEPRECO 2006). However, in the supratidal zone one can still (as of 2009) see exposed rocky areas and boulder beaches with weathered fuel oil stains in the form of asphalt pavements showing trace, sporadic, or fragmentary residues that are resistant to water washing and require the use of shoreline cleaning agents (SCAs).

SCAs are used to increase the efficiency of water washing on substrates polluted with weathered oil, and their use is a trade-off between effectiveness and toxicity. When their toxicity is evaluated in natural environments, the main parameter to be considered is the ecological risk. Suter (1993) defined risk assessment as the process of assigning magnitudes and probabilities to adverse effects arising from human activity or natural catastrophes. In contrast, Bartell et al. (1992) defined ecological risk assessment as the use of available ecological and toxicological information to estimate the likelihood of an undesired event occurring.

The toxicity of a compound in marine invertebrates is normally assessed in early life stages in terms of embryogenesis, with early larval growth, survival, and morphological abnormalities as the end points (His et al. 1999). Early life stages are more sensitive than adult stages and are a critical period in the life cycle of an organism. Sea urchins (Fernández et al. 2006) and molluscs (Beiras and His 1994) are among the organisms most frequently used in embryo–larval bioassays. Recently, a method has been developed for toxicological evaluation in seawater with neonates of the mysid *Siriella armata*, which shows a high

D. Rial (✉) · R. Beiras
Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, r/ Colexio Universitario s/n, 36310 Vigo, Galicia, Spain
e-mail: diegorialconde@uvigo.es

J. A. Vázquez · M. A. Murado
Grupo de Reciclado e Valorización de Residuos, Instituto de Investigacións Mariñas (CSIC), r/ Eduardo Cabello, 6, 36208 Vigo, Galicia, Spain

sensitivity compared with *Daphnia magna* (Pérez and Beiras 2010).

CytoSol, a product selected in the present work due to its efficacy in dealing with weathered fuel oil from the *Prestige*, is a solvent-based SCA (Walker et al. 1999). It does not contain surfactants and is composed mainly of fatty acid methyl esters, with a small proportion of bioremediation enhancers. It mixes with the petroleum and decreases its viscosity (Walker et al. 1999). The mixture released is highly hydrophobic, tending to coalesce and increase the recovery rate. The solubility values in seawater found for CytoSol in the literature range from 7 ppm in seawater (United States Environmental Protection Agency [USEPA] 2009a) to 230 ppm at 18°C (Walker et al. 1999).

The ultimate aims of the present work were to evaluate the toxicity arising from the use of CytoSol and to propose criteria for decreasing the impact associated with its use in cleaning supratidal rocks impregnated with fuel oil from the *Prestige*. First, we carried out a toxicological study of CytoSol as well as the combination of CytoSol plus a crude oil. We then carried out a pilot-scale treatment in the natural environment and evaluated the toxicity of the runoff water. Finally, we characterized the environmental risk associated with the use of CytoSol. To do this we developed a simple spill dilution model that can be used to determine the predicted environmental concentration (PEC) according to the area treated. The toxicological information obtained from the pilot treatment was used to calculate the predicted no-effect environmental concentration (PNEC) and the PEC/PNEC ratio as an expression of the risk.

Material and Methods

Experimental Solutions

CytoSol was obtained from CytoCulture International Inc. (CA, USA). Other chemicals were purchased from Sigma (St. Louis, MO, USA). In the first type of assay (*C_{ULT}* [CytoSol prepared with Ultra-Turrax]), a stock solution of 100 µL/L CytoSol was prepared by direct injection of 100 µL into 1 L of 0.22 µm-filtered seawater (FSW; pH 8.32, O₂ 8.2 mg/L, S 34.2‰, and T 20°C) in a 2-L bottle and emulsification using an Ultra-Turrax homogenizer (USEPA 2009a). Experimental concentrations were chosen on the basis of preliminary trials. The experimental concentrations tested (in the interval 0.5 to 100 µL/L) were obtained by dilution of the stock in FSW.

In the second type of assay (*L&C* [joint effect of Lybian light crude and CytoSol]), 50 mL Lybian light crude oil and 50 mL CytoSol were added to 900 mL FSW in a 2-L flask with a Teflon cap, and the mixture was kept in the dark with orbital shaking (150 rpm) for 48 hours at 20°C. The aqueous

phase (chemically enhanced water-accommodated fraction) was separated in a separatory funnel and used to obtain the FSW dilutions to be tested (in the interval 1 to 1000 mL/L).

For the pilot cleaning *in situ* (Playa de Moreira, Muxía, NW Iberian Peninsula; Fig. 1), the CytoSol was atomized onto the rocky substrate at a dose of 0.25 L/m² and after 5 hours water washing was carried out at ambient temperature (approximately 50 L/m² seawater), creating an aqueous emulsion whose toxicity was evaluated with the third assay (*C_{PILOT}* [pilot cleaning assay]). During the treatment, it was observed that when the runoff remained at rest, a higher phase of fuel oil residue became separated from a lower phase of homogenous, stable emulsion. For toxicological evaluation, the runoff samples were agitated by orbital shaking for 16 hours, followed by separation of the phases and dilution in FSW for testing (in the interval 0.1 to 500 mL/L). In the mysid bioassay, five solutions were carried out (in the range 0.5 to 200 mL/L).

Chemical Analysis

The aqueous phase of the runoff was extracted twice in a separatory funnel with 0.20 volumes of dichloromethane each time, and the extract was concentrated in a rotavap at 40°C and slightly reduced pressure until an oily residue was obtained and quantified gravimetrically (constant weight in a desiccator with KOH at ambient temperature). The residue was extracted 5 times by centrifugation with approximately 10 volumes of pentane each time; the insoluble residue was taken as the asphaltene fraction and

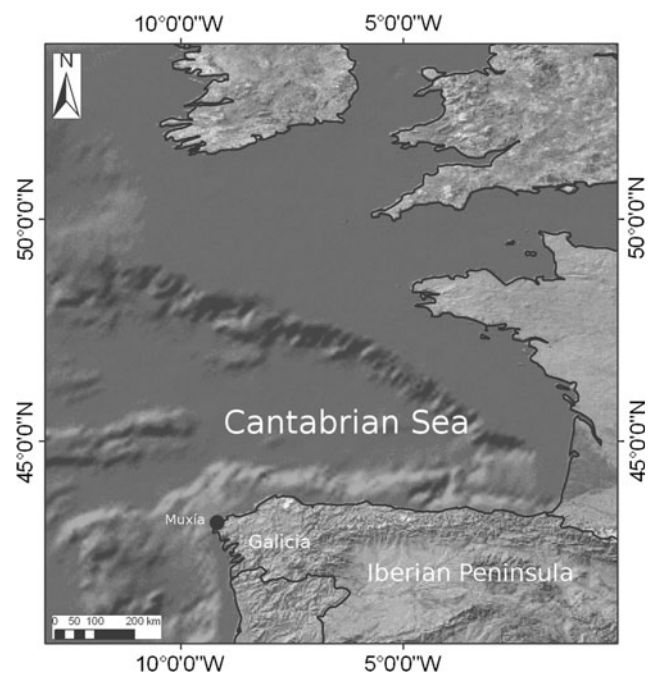


Fig. 1 Location map of Moreira (Muxía, NW Iberian Peninsula)

the extract as the deasphalted fraction. The asphaltenes were quantified gravimetrically, and the deasphalted fraction, which contains low levels of hydrocarbons together with the methyl esters of CytoSol, was analyzed by conventional methods of gas chromatography coupled with mass spectrometry (GC-MS) (Daling and Faksness 2002) in an HP 5850 gas chromatograph equipped with an HP 5971 series J mass selective detector (scan range of 40 to 450 m/z and an HP-5MS column of 60 m \times 0.25 mm). The temperatures were 300°C (injector) and 280°C (detector), and a programme ranging from 40°C (1 minute) to 300°C (20 minutes) with a ramp of 6°C/min was applied. Composition of the runoff is shown in Table 2.

A similar previous treatment with CytoSol showed that the esters of the product are transferred to the deasphalted fraction. The chromatographic profile of the components of CytoSol can be used to quantify their concentration in the deasphalted fraction through the sum of their signals, whose quantitative relations are practically unaltered. The rest of the deasphalted fraction corresponded mainly to the unresolved complex mixture, which was estimated by the difference between the deasphalted fraction and the chromatographic quantification of CytoSol.

Bioassays

Mussel Bioassay

In accordance with the method of Bellas et al. (2008), mature individuals of *Mytilus galloprovincialis* were induced to spawn by thermal stimulation at 25°C in sterile FSW (ultraviolet), and the quality of the gametes (ovum sphericity and sperm mobility) was checked under the microscope. The eggs of a single female animal were transferred to a 50-mL graduated cylinder with FSW, 1 mL sperm was added, and the mixture was shaken gently for approximately 2 minutes to facilitate fertilization. After 15 minutes, the fertilized eggs were transferred to vials with 10 mL FSW dosed with the product to be assayed. Each vial received 400 eggs, and each dose was performed in quadruplicate (the control was performed in quintuplicate). The percentage of fertilized eggs was determined at 70 minutes, in four samples, by counting those showing the first division or presence of a polar body.

The eggs were incubated in the dark at 20°C for 48 hours, after which the larvae were fixed by adding 0.2 mL 40% buffered formalin. In samples of 100 individuals, larvae with a convex hinge, incomplete shell, or protruded mantle were considered abnormal (His et al. 1997). The response was quantified as Eq. 1:

$$R_i = 1 - \frac{N_i}{N_0} \quad (1)$$

where N_0 and N_i are the mean proportions of normal larvae in the control and the i th dose, respectively.

Sea Urchin Bioassay

In accordance with the method of Fernandez and Beiras (2001), the gametes of *Paracentrotus lividus* were obtained by dissection of a couple of adults, and their maturity (ovum sphericity and sperm mobility) was checked with a microscope. The ova were transferred to a 100 mL graduated cylinder containing seawater, approximately 10 microlitres of the sperm taken from the male gonad were added through a Pasteur pipette, and the mixture was 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 minutes, the fertilized eggs were transferred to vials with 10 mL FSW dosed with the product to be tested. Each vial received 400 eggs and each dose was performed in quadruplicate (the control was performed in quintuplicate).

The eggs were incubated in the dark at 20°C for 48 hours, and the larvae were fixed by adding 0.2 mL of 40% buffered formalin. In each vial the maximum length of 45 individuals was measured using an inverted microscope and Leica QWIN image analysis software, version 3.4.0. The corresponding response was quantified as Eq. 2:

$$R_i = 1 - \frac{\Delta L_i}{\Delta L_0} \quad (2)$$

where ΔL_0 and ΔL_i are the mean length increases in the control and the i th dose, respectively.

Mysid Bioassay

In accordance with the method of Pérez and Beiras (2010), neonates released from the marsupium (<24 hours) of a breeding female *S. armata* collected in the natural environment were deposited individually in vials with the solutions to be tested; 20 replicates were performed in all cases. The neonates were incubated for 96 hours at 20°C, with a 16:8-hour light-to-dark photoperiod (light intensity: 20 $\mu\text{E}\cdot\text{m}^{-2}\text{ s}^{-1}$), and fed every 24 hours with 10 to 15 *Artemia salina* nauplii. The response was quantified in terms of mortality rate at 96 hours.

In the three types of bioassay, we used 25-mL glass vials with a Teflon cap, treated at 450°C for 8 hours, kept for 24 hours in 10% HNO_3 (v/v), degreased with a detergent, and washed with distilled water.

The toxicological evaluation of CytoSol (C_{ULT} assay) was carried out through sea urchin and mussel bioassay. The evaluation of the combination of oil and CytoSol

(L&C assay) was carried out through sea urchin bioassay. The assessment of the runoff (C_{PILOT} assay) was carried out through sea urchin, mussel, and *S. armata* bioassay.

Numerical Methods

The dose-response relations were described using the modified Weibull model (Murado et al. 2002) (Eq. 3):

$$R = K \left\{ 1 - \exp \left[- \ln 2 \left(\frac{D}{m} \right)^\alpha \right] \right\} \quad (3)$$

where R is the response (with K as the maximum value), D is the dose of the agent, m is the dose corresponding to the semimaximum response (inhibitory or lethal, according to the case: EC_{50} or LC_{50}), and α is a shape parameter related to the maximum slope of the response.

Fitting procedures and parametric estimations were performed by minimization of the sum of quadratic differences between experimental and model-predicted values using the nonlinear least-squares (quasi-Newton) method provided by the “Solver” macro of the Microsoft Excel spreadsheet. Parametric estimates were confirmed in the nonlinear section of the Statistica 6.0 software (StatSoft Inc., Tulsa, OK, 2001), which was also used to calculate the parametric confidence intervals and model consistency (Student t and Fisher’s F tests, respectively, in both cases with $\alpha = 0.05$).

To directly obtain (with Statistica 6.0) the confidence intervals of doses with responses differing from the semi-maximum response, Eq. 3 was reparametrized to make explicit the corresponding dose. For example, for the dose EC_{10} with the response equivalent to 10% of the maximum, we show $R = 0.1 K$ in Eq. 4:

$$0.1K = K \left\{ 1 - \exp \left[- \ln 2 \left(\frac{EC_{10}}{m} \right)^\alpha \right] \right\} \quad (4)$$

which, with a little algebra, leads to Eq. 5:

$$R = K \left\{ 1 - \exp \left[\ln 0.90 \left(\frac{D}{EC_{10}} \right)^\alpha \right] \right\} \quad (5)$$

The maximum no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were established through analysis of variance and Dunnett’s post-hoc test using the application SPSS v.15.0.1 software (SPSS Inc., IL, USA). In the case of the mussel, the proportions of abnormal larvae were processed by arc-sine of the square root transformation for the analysis of normality and homoscedasticity, and NOEC and LOEC were calculated by nonparametric Kruskal-Wallis and Mann-Whitney U tests if any of those conditions were violated. In the runoff water (C_{PILOT} assay), the toxic units (TUs) were determined according to the expression $TU = 100/EC_{20}$ (Beiras and Saco-Alvarez 2006).

Dilution of the Emission and Risk Analysis

In the C_{PILOT} assay, we used $250 \text{ mL} \cdot \text{m}^{-2}$ CytoSol and $50 \text{ L} \cdot \text{m}^{-2}$ seawater for water washing. A simple model applicable to ecological risk analysis can be defined in the following terms (Fig. 2):

1. The volume of runoff released during treatment of a coastal strip of dimensions $f \times b$ is shown in Eq. 6:

$$E = 50 \times f \times b \times p, \quad (6)$$

where E is the volume of runoff (L), f is the front of the area treated (m), b is the rear of the area treated (m), and p is a value assigned according to distribution of the area affected by the fuel oil (Table 1).

2. If on both sides of the treated strip an untreated strip with a front s is left, it can be accepted that the volume of coastal water affected by the runoff at a given distance from the coast is as follows (Eq. 7, Fig. 3):

$$V = \frac{1}{2}(f + 2s) \times d^2 \times \tan \alpha, \quad (7)$$

where V is the volume affected by the runoff (m^3), d is the distance from the shoreline of the front of the affected volume (m), s is the safety semifront (m), and α is the littoral slope (in degrees).

The s strips can be treated after a reasonable period of time, depending on the biodegradability of the effectors involved. Although we found no data on the biodegradability of CytoSol, the product can be considered as similar to a biodiesel oil, which is also a mixture of methyl esters of vegetable oils. Zhang et al. (1998) stated that methyl esters of soya and rapeseed oil biodegrade 85.5% and 88.5%, respectively, in 28 days. In reasonable agreement with these values, the results of Sendzikiene et al. (2007) showed that biodegradation of methyl esters of rapeseed and linseed oil was 91.2% and 98.3%, respectively, in 21 days. Although more recalcitrant, hydrocarbons show a low level of accumulation: after the *Aegean Sea* spill, the high initial concentration of polyaromatic hydrocarbons in raft-cultured mussels decreased with first-order kinetics

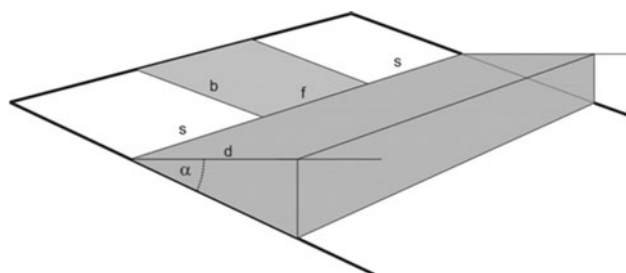
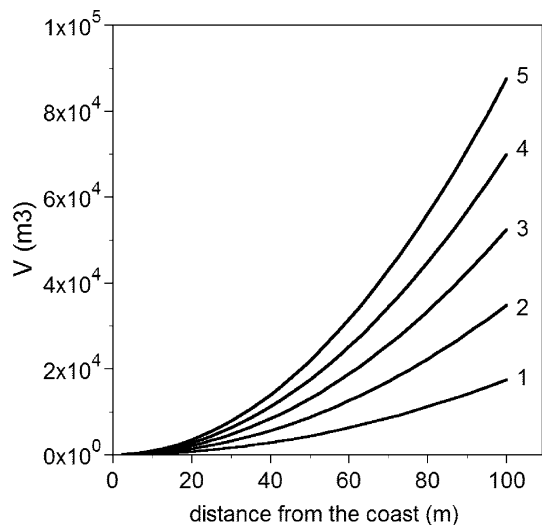


Fig. 2 Dimensions considered in the definition of the criterion for applying CytoSol (see text for details)

Table 1 Description of the surface distribution affected by fuel oil and p value assigned in Eq. 6

Name	Surface (%)	Value
Continual	91–100	1
Intermittent	51–90	0.9
Fragmentary	11–50	0.5
Sporadic	1–10	0.1
Traces	<1	0.01

**Fig. 3** Volume affected according to the distance from the shoreline, for $f = 50$, for $s = 75$, and for the five values of the angle α shown in the figure

and a half-life of 22 to 24 days (Murado 2003). Thus, a reasonable period between the treatment of the f and s strips could be 30 days.

- The predicted concentration of the runoff (PEC) in the volume affected is given by the quotient $PEC = E/V$ ($L/m^3 = mL/L$). The concentration of the runoff at a given distance from the shoreline after a given time, or the mean quadratic distance covered by the emission in a given time, could be estimated by Fick's second law of diffusion. However, the swell increases the rate of dissemination considerably, so Fick's law would involve a strong underestimation of the volume affected in a given time, and the local differences in the currents prevent generalizations based on a detailed diffusion model. Under these conditions, because the runoff is an isolated input of aqueous suspension, a simple but realistic assumption would be that diffusion to distances of 50 to 100 m from the shoreline takes place in times of the order of very few hours, i.e., probably in one tide cycle.
- The proposed application, which is highly localized, involves simply two (at most four) separate emissions

at 30-day intervals. Under such conditions, chronic effects associated with the persistence of the components of the runoff can be ruled out. Therefore, the PNEC was calculated with less strict precautionary criteria than those involved in the factors proposed in the references (Organisation for Economic Co-operation and Development [OECD] 1992; European Commission 2003), which seem to be based on the assumption that the toxicological data are far less precise than those obtained in this work. We proceeded as follows under these conditions:

- We took the lowest NOEC/EC₁₀ obtained in the C_{PILOT} assay.
 - We obtained bibliographic data (Bellas et al. 2005; Beiras and Bellas 2008; USEPA 2009b) on the EC₅₀ of a reference toxic compound (sodium dodecyl sulphate [SDS]) in relation to several estuarine and seawater species and calculated the geometric mean for the available data of each species.
 - Values corresponding to the sensitivities of the different species to SDS were adjusted to a log-normal distribution (Van den Brink et al. 2006), and the median was made to coincide with the lowest NOEC/EC₁₀ obtained in the C_{PILOT} assay.
 - From this curve, we calculated the hazardous concentration to 5% of the species (HC₅), which was taken as the PNEC.
- In accordance with the standard criteria, the calculated risk for a specific scenario is given by the PEC/PNEC ratio, considering that a value <1 represents no environmental risk. Thus, the PEC/PNEC value adopted can be used to estimate the treatable front f for a strip depth b as well as the safety semifronts s at both sides of the treated strip to obtain a tolerable concentration in the volume affected at a given distance d from the shoreline.

Results and Discussion

Chemical Composition of CytoSol

The main products of CytoSol obtained by GC-MS were methyl esters of the following fatty acids: hexadecanoic (palmitic) acid, octadecanoic (stearic) acid, 9-(z)-octadecanoic (oleic) acid, 9,12-(zz)-octadecadienoic (linoleic) acid, and 8,11-(zz)-octadecadienoic acid. Although the declared composition of CytoSol includes minor proportions of other products that enhance bioremediation (Walker et al. 1999), it was not possible to identify them.

C_{ULT} Assay

The EC_{50} obtained for the sea urchin ($r = 0.999$, $p < 0.0001$) was 11.5 $\mu\text{L/L}$ (95% confidence interval [CI] 10.7–12.4) compared with 8.0 $\mu\text{L/L}$ (CI 7.7–8.3) for the mussel ($r = 0.999$, $p < 0.0001$). The EC_{10} was 3.7 $\mu\text{L/L}$ (CI 3.3–4.2) in sea urchin compared with 4.7 $\mu\text{L/L}$ (CI 4.3–5.2) in mussel, and the NOEC was 5 $\mu\text{L/L}$ in sea urchin compared with 2 $\mu\text{L/L}$ in mussel (Fig. 4). The sensitivity to CytoSol was greater in mussel according to the calculated EC_{50} and NOEC values but not for the EC_{10} value. It should be noted that emulsions of CytoSol in seawater obtained by agitation at concentrations higher than approximately 500 ppm were relatively unstable, showing the formation of supernatant microdrops within a few minutes.

The acute toxicities of CytoSol determined in this work ($EC_{50}/48\text{-hours}$ values 8.0 $\mu\text{L/L}$ in *M. galloprovincialis* and 11.5 $\mu\text{L/L}$ in *P. lividus*) contrast with the far more moderate values found for the same product by Walker et al. (1999) working with *Menidia beryllina* (578 to 738 ppm) and juveniles of *Mysidopsis bahia* (121 ppm).

Although we have not found data on CytoSol using the species studied herein, the information on other SCAs suggests that such differences are due to the different sensitivities of the biological entities used in the assays. Indeed, with the product PES-51, the $EC_{50}/48\text{-hour}$ values determined by Shigenaka et al. (1994) were 9.6 (*M. edulis*), 18.7 (*Crassostrea gigas*), 665 (*Artemia sp.*), and 1425 ppm (*Fundulus heteroclitus*).

Similarly, with the dispersant Corexit 9527, Singer et al. (1990) found $EC_{50}/48\text{-hour}$ values of 1.6 to 2.2 ppm (*Haliotis rufescens*) and $EC_{50}/96\text{-hour}$ values of 4.3 to 7.3 ppm (juveniles of *Holmesimysis costata*) and 25.5 to 40.6 ppm (larvae of the fish *Atherinops affinis*). Thus, according to Beiras and Bellas (2008) and Bellas et al. (2005), the toxicity of CytoSol can be characterized as

moderate compared with that of a reference surfactant such as SDS, with an $EC_{50}/48\text{-hour}$ values of 2.4 mg/L (*M. galloprovincialis*) and 4.3 mg/L (*P. lividus*).

These results suggest, furthermore, that in the evaluation of ecological risk associated with the use of SCAs, it is important to include mollusc embryos among the biological entities subjected to bioassay due to their greater sensitivity to this type of formulation. Specifically, one should include those of the genus *Mytilus sp.*, which are widely distributed and of great ecological and economic importance.

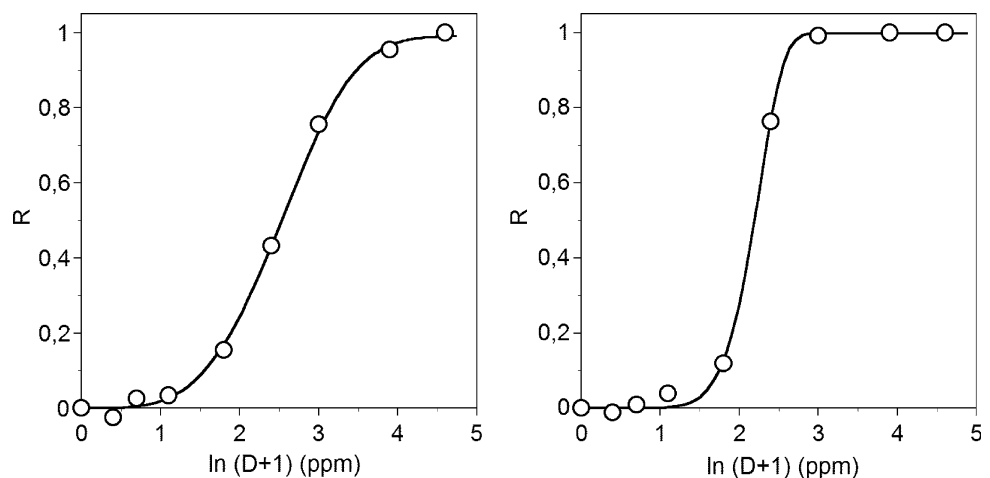
L&C Assay

The EC_{50} obtained for the sea urchin ($r = 0.992$, $p < 0.0001$) was 23.1 mL/L (CI 15.7–33.7) (Fig. 5), and the EC_{10} was 3.8 mL/L (CI 2.3–6.0). The NOEC (5 mL/L) and the LOEC (10 mL/L) were also calculated.

Chemical Composition of the Runoff Water

Of the total concentration of effectors released in the runoff (Table 2), 49.7% corresponded to CytoSol and 50.3% to the hydrocarbons. The contribution of CytoSol was high in both phases (46% in the aqueous phase and 51% in the decanted residue of fuel oil). The concentration of CytoSol determined analytically was 1.64 g/L, which involves a recovery of 44% in the runoff. The losses were related to amounts that were blown away by the wind during the atomization and the waiting period, that slid down sloping rocks during the same period, and that adhered (absorption and adsorption) to the surfaces over which the runoff flowed. Because of the degradability of CytoSol and the nature (rocks, soil) of the substrates that retain it, this fact can be considered to depress the risk associated with its use. According to photographs of the rocks before and after the treatment (Fig. 6), its efficacy was estimated at approximately 80%.

Fig. 4 Inhibition of larval growth in sea urchin (left panel) and enhancement of morphological anomalies in mussel larvae (right panel) by the preparation of CytoSol agitated with Ultra-Turrax in seawater (*C_{ULT}* assay). Dose shown in $\mu\text{L/L}$



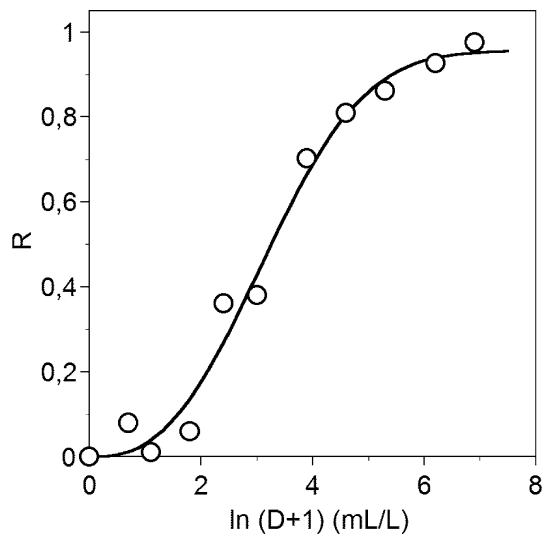


Fig. 5 Inhibition of larval growth in sea urchin by a mixed aqueous extract of Lybian crude oil and CytoSol accommodated together in seawater (*L&C* assay)

Table 2 Composition of the runoff obtained in the treatment of Moreira

Runoff	Mass (g L ⁻¹)	% of total	Relative % per phase
Residue of fuel-oil decanted	2.444		
Asphaltenes	0.460	13.91	18.82
Deasphalted fraction	1.984		
CytoSol	1.245	37.66	50.94
Hydrocarbons	0.739	22.35	30.24
Residue in the aqueous emulsion	0.862		
Asphaltenes	0.159	4.81	18.45
Deasphalted fraction	0.703		
CytoSol	0.397	12.01	46.06
Hydrocarbons	0.306	9.26	35.50
Total	3.306		

C_{PILOT} Assay

The toxicity of the emulsion descended in the order mussel > sea urchin > mysid. In the sea urchin (Fig. 7), the EC_{50} obtained ($r = 0.997$, $p < 0.0001$) was 129.0 mL/L (CI 113.0–147.3); the EC_{10} was 45.0 mL/L (CI 35.9–56.3), and the NOEC and LOEC values were 10 and 20 mL/L, respectively. In the mussel, the EC_{50} ($r = 0.990$, $p < 0.0001$) was 64.3 mL/L (CI 37.8–109.0); the EC_{10} ($r = 0.990$, $p < 0.0001$) was 21.1 mL/L (CI 7.8–54.5), and the NOEC and LOEC values were 10 and 20 mL/L, respectively. In the mysid, in which the mortality at the highest concentration was 20% ($r = 0.967$, $p = 0.003$), only the LC_{10} of 73.0 mL/L (CI 44.4–119.7) was calculated. The TU value for the sea urchin was 15.4.

After an experimental application of oil in a sandy beach mesocosm, an ageing period, and a final treatment with CytoSol and Corexit 9580, Fuller et al. (2000) found that the toxicity to *C. virginica*, *Sheepshead minnows*, and *Cyprinodon variegatus* was low due to “the lack of observed responses with the water column organisms during the oil only application and the oil plus shoreline treatments.” In the present study, the toxicities found in the *C_{PILOT}* assay were not insignificant, particularly compared with those for the mussel larvae. However, the TU value calculated for the runoff with sea urchin (15.4) was lower than that reported by Beiras and Saco-Álvarez (2006) in the first days after the *Prestige* spill in a sandy cove (646) but greater than that measured in the same cove 2 weeks later (1.8), and so forth.

The level of CytoSol detected in the runoff water used in the bioassays of the *C_{PILOT}* assay (397 mg/L [448 μL/L]) indicates that a large part of the toxic response is due to this formulation. In fact, if the toxicity of the preparation were due only to CytoSol, the EC_{50} values that should be attributed to the product would be 27.4 and 58.4 μL/L, respectively, for mussel and sea urchin, whereas those determined in the *C_{ULT}* assays were 8.0 and 11.5 μL/L. It has been reported that petroleum emulsions in water are stabilized by the presence of resins and asphaltenes (Sullivan and Kilpatrick 2002), as well as the presence of silica particles (Binks and Lumsdon 1999), and colloidal materials (Aveyard et al. 2003). The lower toxicity of the runoff is therefore probably due to the sorption properties of the materials that it contains. It is thus reasonable to assume that the high proportion of asphaltenes detected in the emulsion may depress the bioavailability of the most toxic components. Indeed, although very recalcitrant, asphaltenes have a low toxicity because their molecular size prevents them from penetrating the cellular membranes. However, they can retain hydrophobic molecules, such as the hydrocarbons and esters present in CytoSol, thus decreasing the proportions needed to effectively reach the receptors of the target biological entities.

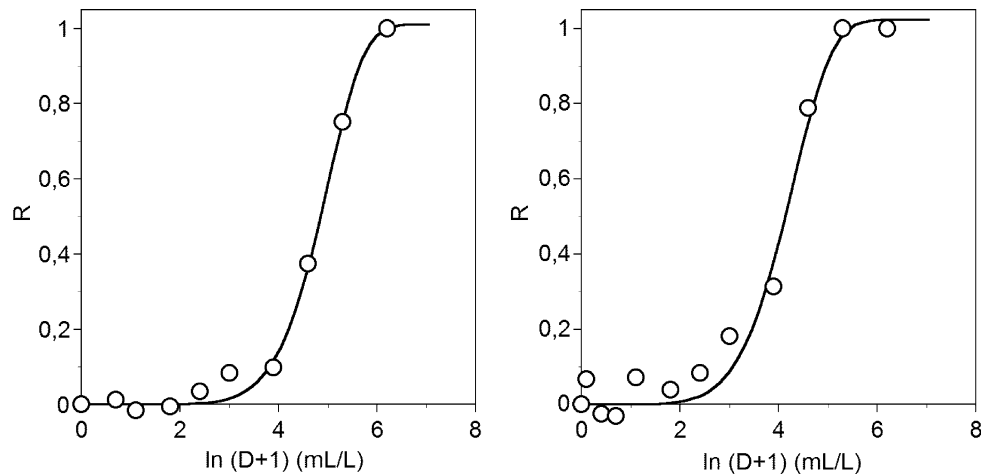
The lack of relevance between laboratory-scale experiments and the field situation has been stated repeatedly (e.g., Linden et al. 1987). Although in our case the comparison between the toxicities detected in the *L&C* and *C_{PILOT}* assays was not direct, the convergence between the results is reasonable. Indeed, taking into account the doses in both assays and disregarding the difference between weathered fuel oil and crude oil, it could be concluded that in the runoff an $EC_{50} < 23.1$ mL/L could not be detected. Thus, the real values (64.3 mL/L in mussel and 129.0 mL/L in sea urchin) agree well with this deduction, particularly if one keeps in mind the fraction of CytoSol that is not transferred to the runoff.

Fieldhouse (2008) stated that the dispersions produced by treatment of Bunker C fuel with CytoSol and cleaning at

Fig. 6 Detail of a boulder before (left photograph) and after (right photograph) treatment with CytoSol under the conditions specified in the text



Fig. 7 Inhibition of larval growth in sea urchin (left panel) and enhancement of morphological anomalies in larvae of mussel (right panel) by the aqueous emulsion from the pilot-scale treatment carried out in the field (C_{PILOT} assay)



25°C with water having a salinity of 3.3 ‰ are moderately stable because they are maintained as resurfaced oil with a low dissipation rate: “capturing the bulk of the oil as resurfaced oil within a boom after 15–30 minutes dissipation is unlikely.” Page et al. (2001) applied CytoSol to a mixture of coastal no. 6 fuel oil and Exxon no. 6 fuel oil in a mesocosm and observed that coalescence took place; the (supernatant) slick was homogeneous and could be picked up easily with a skimmer. As previously stated, in the runoff generated in our treatment the higher phase that separated was also easy to pick up.

Because of the slow degradation of the slicks of weathered fuel oil in the supratidal environment, plus the near uselessness of bioremediation or water washing in this state, and the efficacy with which the use of CytoSol allows cleaning, it seems inevitable to posit the dichotomy between the application of an SCA with a small, isolated ecological impact and the absence of intervention involving a sustained impact on the landscape (albeit a low ecological impact).

For the calculation of the PNEC we considered the option of using the assessment factors proposed by the OECD (1992) and the European Commission (2003), but we decided that they were too high for the proposed treatment. The OECD monograph (1992) recommends an assessment factor of 1000 for EC_{50} estimated in one or two

aquatic species, which, considering the concentration of CytoSol determined in the runoff, would mean a PNEC of 25 $\mu\text{g/L}$. The Marine Risk Assessment section of the Technical Guidance Document on Risk Assessment (European Commission 2003) proposes an assessment factor of 10,000 for the lowest EC_{50} of three taxonomic groups on three trophic levels (algae, crustaceans, and fish), although it accepts the possibility of decreasing the factor to 100 for intermittent emissions. Intermittent application of the SCA and its rapid dilution in the environment make it advisable to use a moderate assessment factor to calculate the PNEC. In our case, the distribution of sensitivities to the runoff of selected species would be the best method for determining the PNEC. Thus, to balance pragmatism and precaution, we decided to seek a surfactant on which sufficient toxicological data were available to adjust to a sensitivity distribution for a set of relevant species in our conditions and superimpose the values obtained in the C_{PILOT} assay. The log-normal distribution parameters obtained for SDS were as follows: $\mu = 8.11$ (CI 8.07–8.13) and $\sigma = 1.26$ (CI 1.21–1.31), with $r = 0.996$ and $p < 0.0001$ (Fig. 8). The PNEC calculated for the runoff based on the most sensitive species was 1.3 mL/L.

Figure 9 shows the PEC/PNEC ratio as an expression of risk according to the front treated in the conditions

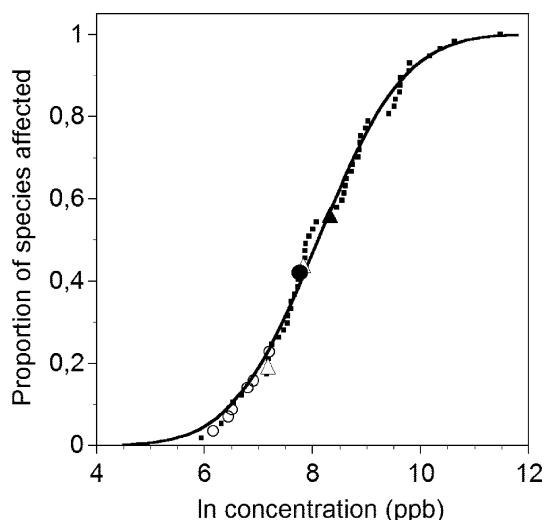


Fig. 8 Distribution of species sensitivity based on acute toxicity (EC_{50} , LC_{50} , and IC_{50}) for SDS extracted from the ECOTOX database, Bellas et al. (2005) and Beiras and Bellas (2008). Embryo–larval tests with molluscs (○), with *M. Galloprovincialis* (●), with sea urchins (△), with *P. lividus* (▲), and with other species and methods (■)

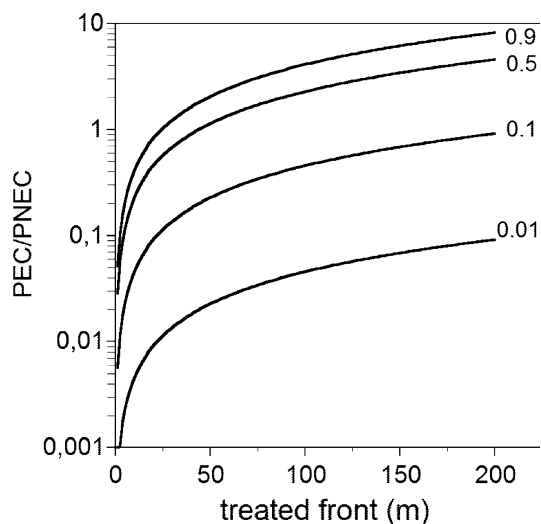


Fig. 9 PEC/PNEC according to the front treated with the following conditions $f + 2s = 200$, $b = 10$, $\alpha = 2^\circ$, $d = 50$, and the four p values noted in the figure

specified in the legend. It should be noted that the dilution model used to calculate the PEC assumes a stagnant volume for reasons of caution and simplicity, although this safety reason is perhaps excessive as we previously commented. However, in the natural environment the mixture can be expected to have a substantially greater volume due to the action of tides, currents, and swell; thus, the concentration will decrease sharply with time, decreasing even further the toxicity associated with release of the runoff.

Conclusion

The mussel, followed by the sea urchin and the mysid, showed the greatest sensitivity to CytoSol and to CytoSol runoff after treatment of a rocky substrate polluted with residues of fuel oil from the *Prestige*. The toxicity of the runoff was moderate, and the precaution adopted in estimating the dilution of this water allowed us to rule out the possibility of the calculated PEC being lower than the real one. Furthermore, intermittent application of the SCA and its rapid dilution in the environment make it advisable to use a moderate assessment factor to calculate the PNEC.

The estimated PEC and PNEC values refer to highly degraded and thin oil slicks. Because the amount of CytoSol used depends on the oil mass per unit area, in a different situation (e.g., rocks impregnated with fresh oil) both the volume of CytoSol required and the toxicity of the runoff will be different. However, we believe that the methodology developed can be generalized to the application of any SCA.

Acknowledgments The authors are grateful to the Centro Para la Prevención y Lucha Contra la Contaminación Marítima y del Litoral (CEPRECO, Ministerio de la Presidencia) for funding this study. We also thank Sara Pérez, Alicia Abalo, Xavier Fraguas, Pilar González, and Araceli Menduña for their incalculable help with the experimental work.

References

- Aveyard R, Binks BP, Clint JH (2003) Emulsions stabilised solely by colloidal particles. *Adv Colloid Interface Sci* 100:503–546
- Bartell SM, Gardner RH, O’Neil RV (1992) Ecological risk estimation. Lewis, Chelsea, MI
- Beiras R, His E (1994) Effects of dissolved mercury on embryogenesis, survival, growth and metamorphosis of *Crassostrea gigas* oyster larvae. *Mar Ecol Prog Ser* 113:95–103
- Beiras R, Saco-Alvarez L (2006) Toxicity of seawater and sand affected by the *Prestige* fuel-oil spill using bivalve and sea urchin embryogenesis bioassays. *Water Air Soil Pollut* 177:457–466
- Beiras R, Bellas J (2008) Inhibition of embryo development of the *Mytilus galloprovincialis* marine mussel by organic pollutants; assessment of risk for its extensive culture in the Galician Rias. *Aquaculture* 277:208–212
- Bellas J, Beiras R, Marino-Balsa J, Fernandez N (2005) Toxicity of organic compounds to marine invertebrate embryos and larvae: A comparison between the sea urchin embryogenesis bioassay and alternative test species. *Ecotoxicology* 14:337–353
- Bellas J, Saco-Álvarez L, Nieto O, Beiras R (2008) Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo–larval bioassays. *Mar Pollut Bull* 57: 493–502
- Binks BP, Lumsdon SO (1999) Stability of oil-in-water emulsions stabilised by silica particles. *Phys Chem Chem Phys* 1:3007–3016
- Centro Para la Prevención y Lucha Contra la Contaminación Marítima y del Litoral (CEPRECO) (2006) Actions to be taken in the event of an oil spill. Ministerio de la Presidencia. Secretaría General Técnica, Madrid, Spain. Handbook (Technical Series), ISBN: 84-7471-133-9

- Daling S, Faksness LG (2002) Laboratory and reporting instructions for the CEN/BT/TF 120 oil spill identification (Round robin test). Nordtest Technical Report no. 499; SINTEF: Trondheim, Norway, pp 1–54
- European Commission (2003) Technical guidance document on risk assessment (part II). European Commission–Joint Research Centre. Institute for Health and Consumer Protection, European Chemicals Bureau
- Fernandez N, Beiras R (2001) Combined toxicity of dissolved mercury with copper, lead and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea-urchin. *Ecotoxicol* 10:263–271
- Fernández N, Cesar A, Gonzalez M, DelValls TA (2006) Level of contamination in sediments affected by the *Prestige* oil spill and impact on the embryo development of the sea urchin. *Cienc Mar* 32:421–427
- Fieldhouse B (2008) Dispersion characteristics of oil treated with surface washing agents for shoreline cleanup. Proceedings of the 31st AMOP Technical Seminar on Environmental Contamination and Response, Calgary, June 3 to 5, pp 373–394
- Fuller C, Bonner J, Dellamea S, Ussery S, Tissot P, Louchouart P (2000) Ecological evaluation of shoreline cleaners used on oiled mesocosm sandy beaches. Proceedings of the 23rd AMOP Technical Seminar on Environmental Contamination and Response, Vancouver, June 12 to 16, pp 795–803
- His E, Seaman MNL, Beiras R (1997) A simplification the bivalve embryogenesis and larval development bioassay method for water quality assessment. *Water Res* 31:351–355
- His E, Beiras R, Seaman MNL (1999) The assessment of marine pollution-bioassays with bivalve embryos and larvae. In: Southward AI, Tyler PA, Young CM (eds) *Advances in marine biology*, vol 37. Academic, London, UK, pp 1–178
- Linden O, Rosemarin A, Lindskog A, Hoglund C, Johansson S (1987) Effects of oil and oil dispersant on an enclosed marine ecosystem. *Environ Sci Technol* 21:374–382
- Murado MA, Gonzalez MP, Vazquez JA (2002) Dose-response relationships: An overview, a generative model and its application to the verification of descriptive models. *Enzyme Microb Technol* 31:439–455
- Murado MA (2003) A desfeita do *Prestige* e os aspetos ambientais da contaminación por petróleo. In: Torres E (ed) *Prestige. ¿A catástrofe que despertou a Galiza?* Candeia Editora, Santiago de Compostela, pp 183–223
- Organisation for Economic Co-operation and Development (1992) Report of the OECD workshop on the extrapolation of laboratory aquatic toxicity data to the real environment. OECD Environment Monographs, 59, Organisation for Economic Co-operation and Development, Paris, France
- Page CA, Fuller C, Autenrieth RL, Bonner JS, Louchouart P, Tissot P et al (2001) Shoreline cleaner use in a sandy beach environment. International Oil Spill Conference, pp 1485–1488
- Pérez S, Beiras R (2010) The mysid *Siriella armata* as a model organism in marine ecotoxicology: comparative acute toxicity sensitivity with *Daphnia magna*. *Ecotoxicology* 19:196–206
- Rousseau C (2003) Accident du *Prestige*, 13 Novembre 2002, Cap Finisterre (Galice), les premières opérations. *Bull d'Inform Cedre* 18:15–20
- Sendzikiene E, Makareviciene V, Janulis P, Makareviciute D (2007) Biodegradability of biodiesel fuel of animal and vegetable origin. *Eur J Lipid Sci Technol* 109:493–497
- Shigenaka G, Yender R, Payton D (1994) Chemistry and environmental effects of the shoreline cleaner PES-51TM. HAZMAT report No. 94–2. Hazardous Materials Response and Assessment Division. National Oceanic and Atmospheric Administration, Seattle, WA
- Singer MM, Smalheer DL, Tjeerdema RS, Martin M (1990) Toxicity of an oil dispersant to the early life stages of 4 California marine species. *Environ Toxicol Chem* 9:1387–1395
- Sullivan AP, Kilpatrick PK (2002) The effects of inorganic solid particles on water and crude oil emulsion stability. *Ind Eng Chem Res* 41:3389–3404
- Suter GW II (1993) Ecological risk assessment. Lewis, Chelsea, MI
- United States Environmental Protection Agency (2009a) National Oil and Hazardous Substances Pollution Contingency Plan (NCP) product schedule notebook. Available at: <http://www.epa.gov/emergencies/docs/oil/ncp/notebook.pdf>. Accessed July 1, 2009
- United States Environmental Protection Agency (2009b) ECOTOX database. Available at: <http://cfpub.epa.gov/ecotox/>. Accessed March 1, 2009
- Van Den Brink PJ, Blake N, Brock TCM, Maltby L (2006) Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. *Hum Ecol Risk Assess* 12:645–674
- Walker AH, Kucklick JH, Michel J (1999) Effectiveness and environmental considerations for non-dispersant chemical countermeasures. *Pure Appl Chem* 71:67–81
- Zhang X, Peterson C, Reece D, Haws R, Moller G (1998) Biodegradability of biodiesel in the aquatic environment. *Trans ASAE* 41:1423–1430