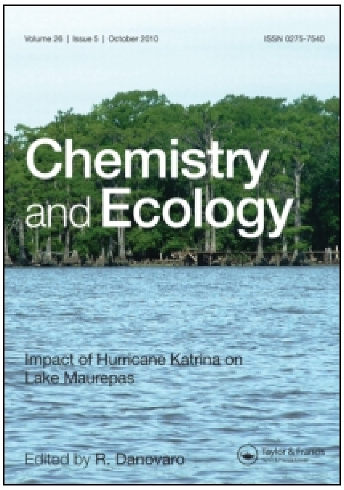

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BRITTLESTARS, BIOMARKERS AND BERYL: ASSESSING THE TOXICITY OF OIL-BASED DRILL CUTTINGS USING LABORATORY, MESOCOSM AND FIELD STUDIES

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Effects of drill cuttings on burrowing brittlestars (*Amphiura chiajei* and *A. filiformis*) were compared at different levels of response, from acute laboratory experiments to chronic field studies. Acute toxicity tests showed that drill cuttings containing oil-based muds had a very low toxicity (LC_{50} 52,800 ppm total hydrocarbons in test sediment) but sub-lethal effects were detected at much lower levels using a variety of biomarkers and assays. Chronic sub-lethal effects were detected around the Beryl oil platform in the North Sea where the levels of oil in the sediment were very low (<3 ppm total hydrocarbons in sediment) and brittlestars were excluded from areas nearer the platform with higher sediment oil content. These results suggest that acute toxicity tests are a poor predictor of chronic response in these animals. The relationship between toxicity at different levels of response and the predictive power of laboratory studies is discussed.

Keywords: Oil drill cuttings; pollution; sub-lethal assays; linking levels of response; ophiuroids

1. INTRODUCTION

As a rule, interest in pollution and the legislation controlling it first developed in terrestrial environments, then in fresh water and lastly in

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marine systems. Our knowledge concerning ecosystem response to pollutants follows a similar pattern of decreasing sophistication as we move into marine environments. There has been a natural tendency to apply risk assessment methods developed originally for use in terrestrial or fresh water systems to marine environments. However, there is increasing evidence that such approaches may be inaccurate, inappropriate and misleading in relation to marine ecosystems (Abel and Axiak, 1991).

Risk assessment in the marine environment is mainly determined through the use of a stepwise system of water-based toxicity tests, principally lethality tests, utilising species chosen to represent a particular trophic level (Lloyd, 1988; Shaw and Chadwick, 1995). Lethality tests are used to produce LC_{50} (the concentration lethal to 50% of the test population) data which are subsequently used to produce a value defined as the Minimum Concentration of a Pollutant (MCP) harmful to aquatic life on which discharge consents are based (Lloyd, 1980). The extrapolation from LC_{50} to MCP has to take into account differences between the test species and native populations, differences between the diluting waters and the receiving waters and the type of toxic action, *i.e.* an accumulating or a non-accumulating toxicant. Each of these variables is allocated a value from which an application factor is derived. The application factor has been called more correctly the 'uncertainty factor', as it is used to represent a link between different levels of response *i.e.* from controlled laboratory-derived data (low level response) to actual effects in the field (high level response) (Forbes and Forbes, 1994). The marine environment is, however, extremely complex and the impact of pollutants on it can be subtle and insidious (Kinne, 1980). The 'uncertainty factor' can be so high and poorly determined as to question seriously the value of current acute toxicity studies in risk assessment.

A specific case is risk assessment associated with drill cuttings. Historically, a major source of oil pollution in the North Sea arises from the dumping of drill cuttings on to the sea bed (Kingston, 1987; GESAMP, 1990; Olsgard and Gray, 1995). These are a complex mixture of drilling muds, emulsifiers, dispersants, stabilisers as well as extracted metals and rock fragments. Drilling muds are frequently oil-based; originally using diesel oil as the base oil but low toxicity oils are now currently used because of the high toxicity of diesel (NSTF, 1993).

The problems in existing risk assessments for drill cuttings discharges are, first an emphasis on the toxicity of the base oil and not on the actual effects of the complex mixture and, secondly, in basing the risk assessment on species of no relevance to the offshore benthic habitat into which the drill cuttings are frequently released. In previous publications (Newton and McKenzie 1995; in press) the use of echinoderms, particularly infaunal brittlestars as habitat-relevant, context-sensitive test species was investigated and discussed. The aim of the current paper is to examine the overall effect of whole drill cuttings from low level responses (laboratory controlled studies) to high level responses (uncontrolled, field studies) to help reveal the predictive limitations of different acute-laboratory based assays and to suggest how best to develop more accurate and robust methodologies for risk assessment in the marine environment.

2. MATERIALS AND METHODS

The ophiuroid, *Amphiura chiajei* Forbes, was collected using a naturalist dredge, from Oban Bay, on the west coast of Scotland. Untreated oil-based drill cuttings were provided by Mobil North Sea Ltd.

2.1. Acute Laboratory Studies

2.1.1. Acute Toxicity Testing (LC_{50} 96h)

The acute toxicity of drill cuttings was assessed by performing a standard LC_{50} 96 h toxicity test. The assay was based on the procedure from Lloyd (1980), including recommendations from Abel and Axiak (1991), and was performed under static water flow conditions with daily renewal of drill cuttings. The concentration of cuttings used was 0 to 100 % cuttings in sediment and was expressed as the concentration of total hydrocarbons (THC) in the sediment. The THC concentration was determined from the oil content of the cuttings (derived from GC analysis) and the proportion of cuttings in the sediment. A shallow substratum of fine, washed sand was provided allowing the brittlestars to burrow. The assays were performed at

15 °C for a period of 96 hours, and control and treatments were tested in triplicate. The brittlestars were examined after 1, 2, 5, 18, 24, 38, 48, 72, and 96 hours. Mortalities were recorded and dead individuals removed from the test containers. Death was defined as no observable movement of arms and tube feet and no recovery and movement following two minutes of aerial exposure. Data interpretation was by probit analysis using probit values generated by Minitab data analysis software. Concentration-mortality curves were constructed by plotting cumulative mortality (transformed to probit values) against toxicant concentration (THC in sediment, log-transformed). Regression of the data was used to produce probit lines and from the regression equation the LC_{50} calculated.

2.1.2. *Acute Sub-Lethal Assays*

The sub-lethal effects of drill cuttings were examined over a four day period, at 96 h. *A. chiajei* were placed in test containers which contained filtered sea water and a shallow layer of sand. The cuttings were mixed with the sand before the addition of the brittlestars. The concentrations of the treatments were 0, 1,200 and 4,800 ppm total hydrocarbons (THC) in sediment. Control and treatments were tested in triplicate. *A. chiajei* were carefully transferred to a replicate set of tanks on a daily basis to maintain concentration of the cuttings.

Throughout the experiment the effects of the cuttings were examined using sub-lethal assays. These included 'burrowing' behaviour and the abundance of symbiotic subcuticular bacteria (SCB). The brittlestar, *Amphiura*, shows a behavioural response to unfavourable environmental conditions by either not burrowing into the sediment or by surfacing from its burrow. Burrowing activity was defined by the number of arms clearly visible on the sediment surface, see Newton (1995 thesis) for assay details. Amphiurid brittlestars contain substantial populations of symbiotic bacteria that are present between the surface coats (cuticle) and the epidermis (Kelly and McKenzie, 1995). The number of these SCB has been shown to change in response to stress and changes in abundance of SCB has been proposed as a biomarker of stress. The tissue loading of SCB was determined by homogenising arm tissues and direct counting using epifluorescent microscopy. The number of SCB were expressed per gramme of tissue wet weight (see Kelly and McKenzie (1992)) and Newton (1995) for assay details).

2.1.3. Chronic Sub-Lethal Tests: Mesocosm Systems

The chronic sub-lethal effects of drill cuttings were assessed under semi-natural conditions using mesocosm experimental systems. Mesocosm systems consisting of 250 1 tanks with pump-ashore sea water flow-through were prepared.

Sediment and brittlestars were controlled from Oban Bay. The tanks were randomly designated as cuttings or control and all treatments were tested in triplicate. Drill cuttings were added to the tanks at two different loadings to create 'light' and 'heavy' treatments of cuttings. The sediment in the 'light' and 'heavy' mesocosms had an initial mean concentration of 118 and 790 ppm THC respectively. The mesocosms were monitored over a six month period, initially every week, then every month. This included measuring the concentration of oil in the sediment and the sub-lethal responses of *A. chiajei*. Sub-lethal stress was assessed using changes in the abundance of SCB (Kelly and McKenzie, 1992; Newton, 1995).

2.1.4. Chronic Sub-Lethal Responses: Field Studies

The long-term effects of cuttings on brittlestars was examined using field studies. The Beryl oil field is located in the British sector of the North Sea and is serviced by two platforms; Beryl Alpha and Beryl Bravo. Both platforms have a long history of using oil-based drill cuttings. Field sampling was performed from the MV *Maersk Rover* along a transect extending NNW and SSE of the Beryl Alpha platform. Sampling sites were located along the transect between 500 m and 7 km north and 250 m to 7 km south of Alpha platform. At each site sediment was collected using a 0.1 m^2 Day grab. Surficial sediment was collected to determine total hydrocarbon contamination. *A. filiformis* were collected from grab samples and analysed for sub-lethal stress by examining the abundance of SCB and induction of NADPH cytochrome c reductase (Newton, 1995).

2.1.5. Linking Levels of Response

The toxicity of drill cuttings was examined at three levels of response; the acute response under laboratory controlled conditions (low); the chronic response under semi-natural conditions (medium); and the chronic response under field conditions (high). The data from each level of response was used to estimate and compare the toxicity of drill cuttings. Risk assessment is usually based on extrapolating from acute laboratory based tests, described below (Lloyd, 1980). Using this standard testing procedure the minimum concentration of cuttings harmful to *Amphiura* was determined.

Acute toxic response(LC_{50}96 h)

⇓

Application Factor(receiving water, type of response, test organism)

⇓

"Minimum Concentration of Pollutant" harmful to aquatic life.

3. RESULTS

The responses of *Amphiura* to drill cuttings at different levels of response are summarised in Table I.

3.1. Acute Toxic Responses

Data from the acute toxicity tests suggest that *A. chiajei* is not sensitive to drill cuttings. The 96 h LC_{50} value was 52,000 ppm THC (Tab. I). At this concentration, the drill cuttings would be classed as having a low acute toxicity (J. Buchanan, pers comm.). There was a good fit between the regression model and probits ($P < 0.001$) and there was no evidence of a split probit line. A bi-phasic probit line can indicate that the test organisms were not responding as one population and that there are resistant individuals within the population. It may also indicate the test population exhibits more than one response to the toxicant *i.e.* the test substance is a mixture of toxicants (Abel and Axiak, 1991). The data suggests that, within the test period, the drill

TABLE I The effects of drill cuttings on *Amphiura* using acute laboratory controlled experiments, chronic semi-controlled mesocosms and chronic field studies

Level of response	*Assay or observation*	*Response*	*Drill cuttings (ppm THC)*	*Predicted or Observed toxicity*
Acute response {laboratory controlled}	LC_{50}	96 h exposure = 50 % mortality	52, 800 ppm	LOW
	Burrowing	96 h exposure = decrease in burrowing activity	4, 800 ppm and 1,200 ppm	LOW
	SCB	96 h exposure = decrease in SCB	4, 800 ppm and 1,200 ppm	LOW
Chronic response {semi-controlled mesocosms}	SCB	2–12 weeks exposure = decrease in SCB	118 ppm and 790 ppm	MEDIUM
Chronic response {field studies}	SCB	years of exposure = decrease in SCB	< 3 ppm	HIGH
	Amphiura excluded from sediments	years of exposure = death/ migration of adults death / poor colonisation/by juveniles	> 10 ppm	HIGH

cuttings were acting as a single toxicant despite being a complex mixture.

Sub-lethal stress was detected following acute exposure to cuttings (Tab. II). Throughout the experiment there was significantly more burrowing under the control conditions compared with the other treatments (Tukey, $P < 0.05$). Exposure to cuttings had a significant effect on the tissue loading of SCB (ANOVA, $P < 0.001$). In both assays the greatest effect was found with the higher concentration (4,800 ppm) of cuttings. At both concentrations, the effect was surprisingly subtle considering the high concentration of cuttings used in the assay (4,800 and 1,200 ppm). The acute sub-lethal data therefore supports the findings of the LC_{50} that drill cuttings have a low acute toxicity.

3.2. Chronic Sub-Lethal Responses

Chronic sub-lethal stress was detected in *A. chiajei* following exposure to low concentrations of drill cuttings using mesocosm systems (see Newton, 1995 for more details). The abundance of SCB was

TABLE II The sub-lethal effects of acute exposure (96 h) of drill cuttings on *A. chiajei*. The tissue loading of Sub-Cuticular Bacteria (SCB) is expressed as number of bacteria per gramme wet weight. Burrowing activity is expressed as the number remaining on the surface. Data are expressed as the mean ± S.E. The values in parentheses represent the percentage of *Amphiura* not burrowing

Treatment	***SCB Assay*** *bacteria per g wwt*	***Burrowing Assay*** *no. on surface*
Control	15.9 ±1.1	6.0±0.58 (60 %)
Cuttings 1,200 ppm	10.3 ±0.9	7.7±0.33 (77 %)
Cuttings 4,800 ppm	9.0±0.85	8.3±0.33 (83 %)

significantly reduced by treatment (ANOVA, $P < 0.001$) and the tissue loading of SCB was significantly higher in the controls compared to either the light (118 ppm) or heavy (790 ppm) treatments (Tukey, $P < 0.05$). Following the initial insult of cuttings (day 0), in both treatments the abundance of SCB showed a gradual decline until 47 d. After this sampling date the abundance of SCB began to show signs of recovery and by 110 d the abundance in both treatments was approximately 70% of control values. The abundance of SCB in the cuttings; treated tanks decreased to approximately 50% of control values and remained at a depressed level throughout the experiment. Therefore, at this level of response, the cuttings could not be classed as having a low chronic toxicity.

A. filiformis were absent from sediments in the proximity of the Beryl oil platforms and were generally found only in sediments at > 2 km from the platforms. The distribution of *A. filiformis* did not appear to be related to sediment type and there was no relationship between median particle size and collection of brittlestars. The distribution did, however, appear to be related to the presence of hydrocarbon contamination as *A. filiformis* were absent from sites with a high concentration of total hydrocarbons. Sub-lethal stress was detected in *A. filiformis* at sites more distant from the platforms. SCB numbers were not closely correlated to the concentration of hydrocarbon contamination of sediment; however, there was a trend of increasing abundance of SCB with increasing distance from Beryl (Newton and McKenzie, unpublished data). This suggests that the effect may be historical and not related to contemporary concentrations of oil, or that the effect was not due solely to the oil content of the cuttings. Due to the exclusion of *A. filiformis* close to platforms and the possible sustained effects, at this level of response drill cuttings could be classed as having a high chronic toxicity.

3.3. Linking Responses

In risk assessment, LC_{50} data (low level responses) are used to produce a value defined as the "Minimum Concentration of a Pollutant" (MCP) harmful to aquatic life (high level effects) based on an 'application factor'. The application factor takes into account the test species, diluting water and the type of toxic action. Each of these variables is allocated a value from which an application factor is derived. Using standard methods described by Lloyd (1980), the application factor for this study would have a value of 100. This is based on a value of 5 for differences between the diluting water and receiving water, a value of 20 for the type of dose - response curve and a value of 1 for the test species. The 'safe' concentration of cuttings would be determined at 528 ppm (see below).

Acute toxic response = 52,800 ppm
(LC_{50} 96 h)
⇓
Application Factor = 100
(receiving water(5), type of response(20), test organism(1))
⇓
"Minimum Concentration of Pollutant" harmful to aquatic life = 528 ppm

4. DISCUSSION

The central problem in risk assessment is balancing the ecological relevance of an assay system with its practicality and cost. Laboratory-based assays are predictive, can be made under controlled conditions, and have little economic cost, but they have been criticised frequently for being of low to minimal relevance to the actual environmental impact of a pollutant (Moriarty, 1983; Forbes and Forbes, 1994; Chapman, 1995). Community analysis can, at least in theory, describe accurately the changes produced by a pollutant in an ecosystem but this is very expensive to do thoroughly. It is also difficult to extrapolate predictably from a specific community analysis, because of the complex variables at play within any environment and the problems in establishing control groups (McIntyre, 1984). The thrust of research must therefore be to maximise the ecological relevance of any assay system while understanding the residual compromises inherent within it. One approach to achieving this is through comparative multi-level response analyses using habitat-relevant species.

The Oslo and Paris Commission have proposed a test system for offshore chemicals/products. Products that are likely to deposit in the sediment must be tested on one alga (*Skeletonema costatum*, 72 h growth inhibition), one herbivore (*Acartia tonsa*, 24 and 48 h lethality test) and one sediment re-worker (*Corophium volutator*, 10 d lethality test) (Weideborg *et al.*, 1997). However, the data produced by the current study indicate that these tests, whilst providing data on comparative toxicities, will not examine in sufficient detail higher level responses, *i.e.* the actual effects in the field. Thus, they will have severe limitations in protecting the marine environment.

Echinoderms are excellent choices as model systems in marine risk assessment for benthic ecosystems being large, relatively sedentary, often of ecological importance, widespread, easy to keep and sensitive to many types of contaminants (Hermelin *et al.*, 1981; Delmas and Regis, 1984; Bowmer *et al.*, 1986; Newton and McKenzie, *in press*). Amphiurid brittlestars fit all of these descriptions and have the added advantage of being infaunal, ensuring direct contact with contaminants present on or in the sediment. Amphiurid species are common in near and offshore sediments in temperate environments worldwide, making it easier to develop assay systems of wide geographic applicability. Community analyses around oil platforms in the North Sea suggest that *A. filiformis* is also extremely sensitive and vulnerable to the effects of oil production (Olsgard and Gray, 1995) and may indeed be the main species in which a direct and seriously detrimental effect of oil production activity can be shown.

The purpose of the current study was to compare the varied responses of amphiurid brittlestars to drill cuttings using a variety of assay systems ranging from acute toxicity tests to field studies with the aim of establishing what assays provide the best balance between practicality and relevance. This is not to suggest that amphiurids should be treated as universal sensitive species but in the context of drill cuttings, and perhaps oil production generally, they appear to be the most appropriate because of their sensitivity and the actual impact upon their populations in the North Sea. The laboratory and mesocosm studies used *A. chiajei* as the test species whereas the field data is derived from *A. filiformis*. Although both species were present in North Sea sediments, the numbers of *A. chiajei* collected were insufficient for analyses. *Amphiura chiajei* and *A. filiformis* both contain symbiotic bacteria and in similar numbers (Kelly and McKenzie, 1995). For the purpose of discussion we will ignore any possible differences in response between these two, closely related species though further studies on *A. filiformis* in laboratory and mesocosm assays will be necessary to determine the extent of any differences between them. What is certain is that *A. chiajei* will be a better model for impact assessment of drill cuttings on *A. filiformis* than any of the traditional model species, such as the shrimp, *Crangon crangon*.

The three levels of response examined produced markedly different evaluations of the predicted risk of drill cuttings. The concentration of cuttings required to cause the death of 50 % of the population within 96 hours is high (52, 8000 ppm) suggesting that the cuttings have a low environmental risk. The mesocosm systems showed that exposure to a low concentration of cuttings (118 ppm) had considerable and prolonged sub-lethal effects. The field studies indicate that drill cuttings do have a significant long-term impact on *Amphiura*. However, the impact may be related to the non-hydrocarbon components of the cuttings such as metals (Ba, Cr, Cu, Pb), physical disturbance, or organic enrichment due to the discharge of cuttings. This demonstrates that at higher level responses the effects of a stressor are difficult to isolate, suggesting that mesocosm systems will be a superior approach to the problem. Mesocosm systems allowed the effects of drill cuttings to be examined under semi-natural conditions, and in isolation from the effects of other contaminants.

Current testing procedures assume that there is a link between acute toxic responses in the laboratory (low level responses) and effects in the field (high level responses) (Forbes and Forbes, 1994). This may be correct in some circumstances but seems incorrect in the case of drill cuttings and amphiurid brittlestars. Drill cuttings are a complex mixture of components, which vary in their toxicity and bioavailability. Therefore, the level at which an assay is performed will determine which components are being tested. An acute test will examine principally the more soluble components whereas a chronic test will examine the less soluble or bioavailable components and allow for additive or synergistic interactions. This study shows that the data derived from higher level studies did not validate the responses observed at the lower level and so the effects of drill cuttings need to be examined at all levels of responses. As noted by Chapman (1995), laboratory tests are carefully controlled experiments that do not replicate field studies. Conversely, field studies do not validate laboratory data but provide additional information on environmental risk and the actual environmental impact. To our knowledge the current study is unique in comparing the impact of drill cuttings on the same invertebrate at different levels of response.

In conclusion, the results of this study suggest that chronic effects of drill cuttings on amphiurids are not well predicted by acute tests and more emphasis should be placed on longer-term, semi-natural experimental systems using habitat-relevant species. It is also important that test conditions match the conditions into which the pollutants are being discharged. Temperature, salinity, water movement and pressure will all alter the toxic action of chemicals and their bioavailability. This will become increasingly important as oil production moves into the deeper waters away from the continental shelf. The complexity of marine ecosystems may dictate the need for a case-by-case approach using the best, habitat-relevant species available for that site.

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