DETERMINATION OF DIOXIN-LIKE ACTIVITY IN SEDIMENTS FROM THE EAST SHETLAND BASIN

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Introduction
Dioxin-like compounds are ubiquitous in the environment[1] and their presence is primarily the result of by-products of human activities including industrial and other sources (e.g. waste incineration)[2]. They enter into the aquatic environment via a number of routes including atmospheric deposition and effluent discharges. Due to their hydrophobic nature sediments are the eventual sink for dioxin-like compounds in the aquatic environment where they provide a source of potential exposure of dioxins and dioxin-like compounds to aquatic organisms[3]. Once present in the food chain, dioxins and dioxin-like compounds bioaccumulate, thus posing a threat to benthic organisms and their predators[4].

At present there is no concerted approach for monitoring of dioxin-like compounds in the marine environment. This is due to the extremely low concentrations at which deleterious effects can be observed. In order to monitor these extremely low concentrations, sophisticated analytical techniques such as high-resolution gas chromatography coupled to high-resolution mass spectroscopy (HRGC-HRMS) are required, which makes analysis expensive. An alternative approach is bio-analytical analysis using a receptor-reporter based cell line. The Dioxin Responsive-Chemically Activated Luciferase Expression (DR-CALUX®) assay utilises a receptor-reporter gene system. The assay is mechanism specific and is based on the interaction of compounds with the arylhydrocarbon receptor (AhR). The assay is not compound specific and produces a response with all compounds capable of interaction with the AhR[5]. The assay allows the integration of all dioxin-like compounds present and produces toxic equivalent concentrations relative to the most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD-TEQ_{CALUX} values) for samples screened. The usefulness of the DR CALUX® screening assay for monitoring purposes was investigated on sediments taken from the East Shetland Basin, an area of intense oil production.

Methods and Materials
Sample collection. Single sediment samples (n=108) were collected by means of a Day grab from sites across approximately 3,000 square miles of the East Shetland Basin between 20th July and 3rd August 2002. The sampling positions of all the sites are shown in Figure 1. **Extraction**. Sediment samples were air-dried and solvent extracted with dichloromethane to produce total extracts. The sample extracts were then transferred into hexane before aliquots assigned for assay. The DR-CALUX® assay was conducted on both the total and cleaned-up extracts. The total extract was screened to ascertain the contribution from both stable and labile dioxin-like compounds. An aliquot of sample extract was also manipulated using a simple clean-up method to ascertain the contribution from stable dioxin-like compounds only, with the cleaned extract dissolved in dimethyl sulphoxide. **DR-CALUX® analysis.** The DR-CALUX® cell line was provided under licence from *Biodetection Systems Bv*., Netherlands. The cell line was constructed as described by Garrison et al.[5]. These cells consist of the rat hepatoma (H4IIE) cell line that has been stably transfected with the luciferase reporter gene (pGudLuc) from the firefly, *Photinus pyralis*. The luciferase activity was determined using an automated luminometer, and interpolated with a TCDD standard curve to produce the TEQ_{CALUX} values for the sample extracts. The DR-CALUX® assay was conducted in accordance with a set of strict quality control (QC) criteria. **Isolation of 'dioxin-like' substances**. An aliquot of each extract underwent a simple clean up procedure to remove all the labile compounds unstable to oxidative breakdown, effectively isolating the most stable compounds. The extracts

were passed through a deactivated silica column that also contained $AgNO_3$ and anhydrous Na_2SO_4. G*eostatistical Interpolation Technique* Some of the large spatial data sets produced underwent a geostatistical interpolation technique to aid their interpretation. The *Kriging Interpolation* method was utilised to produce contour modelling of the data sets.

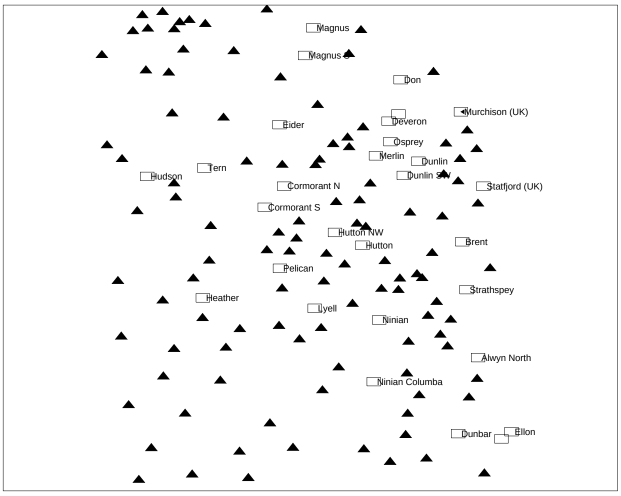

Figure 1. Map showing the positions of all the sampling sites and offshore production platforms within the East Shetland Basin. ▲=Sediment sample site. □=Oil production platform. Sampling area Lat. 60.5 – 61.65 N and Long 0.6 – 1.8 E(Note one degree of Lat. Is 60 miles.

Results and Discussion

Arylhydrocarbon receptor activity (DR-CALUX® Assay).

Activity of Cleaned-up Extracts. The 24 h TEQ_{CALUX} data for all 91 samples ranged from 0.1 pg to 34 pg TEQ_{CALUX} g^{-1} with a mean of 8.5 pg TEQ g^{-1}. As a comparison, the mean activity for stable compounds in a recent survey of UK estuarine sediments[6] was 24 pg TCDD TEQ g^{-1}. DR-CALUX® analysis of a cuttings pile from the Shetland Basin[7] had an activity of 9.2 and 59 pg TCDD TEQ g^{-1} , whilst other studies in the southern North Sea[8] have showed TEQs of between 6 and 21 pg TCDD TEQ g^{-1} .

To put the values in context is difficult, as the UK does not currently have an Environmental Quality Standard (EQS) or Environmental Quality Guideline (EQG) for dioxin compounds in sediments. Other countries including Canada, the USA and the Netherlands have produced dioxin EQGs. The Canadian, American and Dutch EQGs, quoted as TCDD toxicity equivalents, are 1, 2.5 and 13 pg TEQ g^{-1}, respectively [9,10,11]. Using these guideline,s 89 of the 91 samples are above the most stringent (Canadian) EQG and 17 of the 91 samples are above the least stringent (Dutch) guideline value. For the sites breaching these guidelines, it is likely that some harm would be caused to benthic organisms associated with these sediments and also to predator organisms. The results from the Kriging analysis of the stable dioxin-like activity are shown in Figure 2 as a contour map. Areas that breached the least stringent Dutch guideline of 13 pg TEQ g^{-1} are shown above the labelled 14 pg TEQ g^{-1} contour. This area covers approximately 500 square miles (one-sixth) of the area sampled. The compounds responsible for the activity are likely to be dioxins, furans and coplanar chlorobiphenyl congeners, although other compounds may also contribute.

Activity of Total Extracts. The TCDD TEQ_{CALUX} values for the total extracts (n=108) were determined over a 6 h exposure period. The values range from 4.4 ng to 122 ng TEQ g^{-1} with a mean of 36 ng TEQ g^{-1}. As a comparison, the mean activity of total extracts from a recent survey of UK estuarine sediments was 205 µg BaP units g^{-1}. These values are 3 orders of magnitude higher than those found in the cleaned-up extracts. This is not unusual, and due to the removal of PAHs during clean-up, which show a very potent ArH activity compared to 2,3,7,8 TCDD. The Geostatistical Interpolation technique was again conducted on this dataset with the distribution of the total CALUX activity shown in Figure 3.

The vast majority of the dioxin-like activity in the East Shetland sediments is attributable to labile compounds (e.g. PAHs). PAHs are known AhR agonists due to their coplanar configuration, but only produce dioxin-like toxicity if exposure is sustained in an organism since they are relatively quickly metabolised in the cell [12].

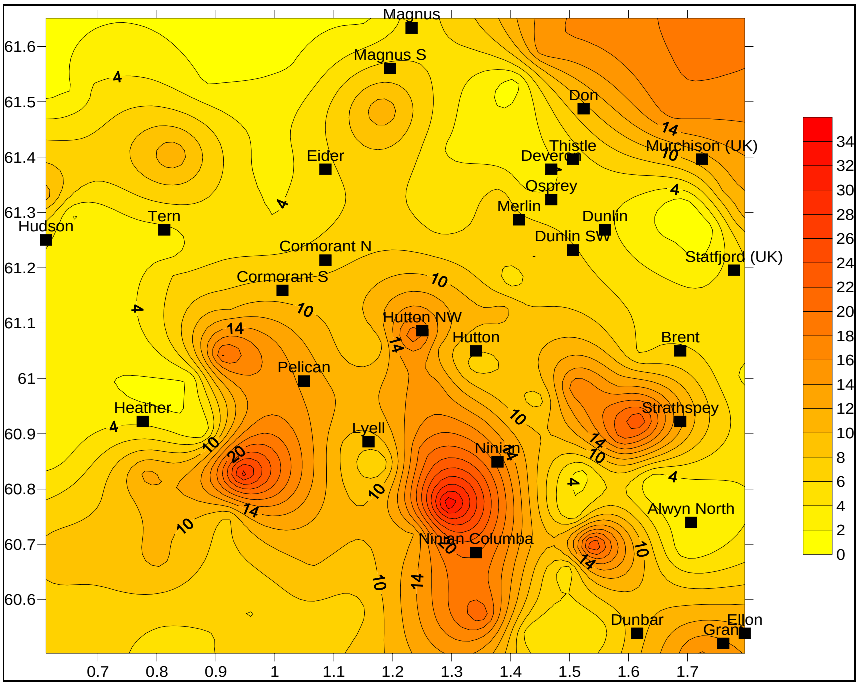

Figure 2. CALUX Assay Screening: Distribution of Dioxin-Like Activity (pg TEQ g^{-1}) Produced from Stable Compounds from East Shetland Basin Sediments using a Geostatistical Interpolation Technique. ■ =Offshore production platform. Sampling area Lat. 60.5 – 61.65 N and Long 0.6 – 1.8 E(Note one degree of Lat. is 60 miles.

Conclusions

The DR-CALUX® assay has been shown to be a useful tool for assessing the distribution and level of activity of sediment-associated contaminants in the East Shetland Basin. The stable dioxin-like, or arylhydrocarbon receptor-based activity was shown to occur at potentially harmful levels in some areas of the East Shetland Basin, according to national guidelines from three countries. Samples, or sites, showing levels of contamination above set action limits would require targeted chemical analyses of a range of known potential candidate compounds to identify the causative compounds. Bioassay-directed fractionation procedures could also be used as the second tier to identify the causative agents.

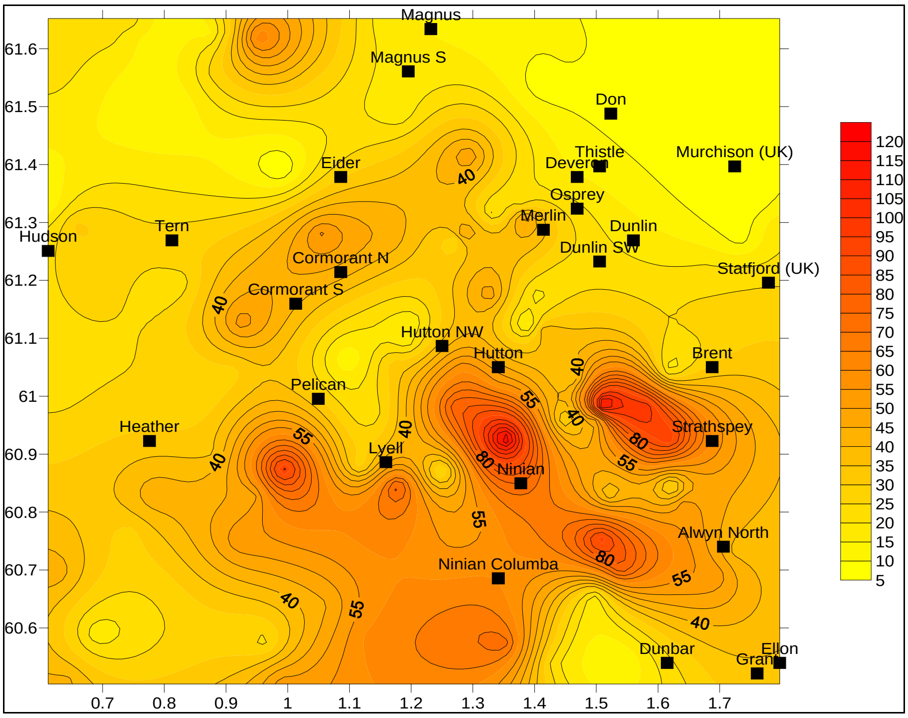

Figure 3. CALUX Assay Screening: Distribution of Dioxin-Like Activity (ng TEQ g^{-1}) Produced from both Labile and Stable Compounds from East Shetland Basin Sediments using a Geostatistical Interpolation Technique. ■ =Offshore production platform. Sampling area Lat. 60.5 – 61.65 N and Long 0.6 – 1.8 E(Note one degree of Lat. is 60 miles.

Acknowledgements

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