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A13–ENVIRONMENTAL TOXICOLOGY: DIAGNOSTICS

Organised by K.C. Thompson, R. Handy, D. Rawson, J. Farmer and J. Wharfe for the Royal Society of Chemistry, Society of the Chemical Industry, Society of Environmental Toxicology & Chemistry UK Branch and the Society for Experimental Biology and sponsored by the Environment Agency, Chemex, AlControl Laboratories, The Royal Society of Chemistry and the Society for Chemical Industry

A13.1
The use of biomarkers and other biological rapid-response tools for monitoring pollution in the aquatic environment

P. Matthiessen, (Centre for Ecology and Hydrology, Lancaster, UK)

There is a need to monitor the aquatic environment for the effects of pollutants because pre-release risk assessments are intrinsically unable to detect all problem chemicals. Furthermore, pollutants can cause mixture effects in unexpected ways which may be impossible to predict. Traditionally, surface waters have been monitored by a rather un-coordinated amalgam of chemical analyses and surveys of invertebrate community structure. This approach is limited by the fact that it is only practical to monitor for a small proportion of the many anthropogenic substances in the environment, and purely chemical data are hard to interpret with precision. Furthermore, using community-level change as the measure of biological impact is a rather crude tool because it cannot avert ecosystem damage and is unlikely to provide diagnosis of the causative substances. The need for the ability to diagnose causes and predict incipient problems led to the development of biomarkers and other biological response tools. These can range from highly pollutant-specific biochemical and cellular changes, to non-specific physiological measures which are able to give forewarning of ecosystem-level damage. This paper describes some of these biomarker techniques and highlights their advantages and drawbacks. It also discusses the need for greater automation of biological monitoring tools.

A13.2
Biological effect measures — a regulatory perspective

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Recent and emerging legislation form Europe together with overarching strategies for whole ecosystem management from Central Government and environmental regulatory agencies in the United Kingdom, focus more on ecological outcomes. In its Vision for the Environment, the Environment Agency for England and Wales aims to achieve a Better Quality of Life and an Enhanced Environment for Wildlife.

To achieve these aims biological effect measures are required to provide a better understanding of ecological relevance and ecosystem protection and to improve risk assessment procedures. Chemical releases, mainly as complex mixtures, represent one of the most important threats to humans and wildlife and perhaps not surprisingly the associated legislation is both numerous and complex. The current legislative approach is limited by the shortfall of information for many chemicals on their toxicity, persistence and ability to bioaccumulate. Less than 1% of commercially available chemicals have sufficient information to derive a quality standard and those that are available fail to recognise additive and enhance effects resulting from complex mixtures.

The development and application of Direct Toxicity Assessment by the Environment Agency, whereby whole samples are measured for their total toxic effect has significantly advance the capability to manage releases of complex effluents. New biomarker methods, including the evolving areas under genomic and proteomic technologies, further our understanding of the mechanistic links between the stressor and the effect and offer the

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potential for both improved understanding and cost-effective, targeted monitoring effort. This presentation reviews current regulatory practice.

A13.3
Biosensors & biomimetic sensors for environmental diagnostics

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Prolific quantities of physical and chemical information are required to feed hypotheses and models in order to resolve the environmental and safety conundrums of modern times. Although conventional chemical analytical procedures have improved considerably with respect to sensitivity, reliability and automation in recent years, they often require sophisticated and expensive instruments operated by skilled personnel. Microanalysis systems such as lab-on-a-chip technology offer one route forward, but chemical analysis offers only limited insight into the biological effects of pollution. Over the past decade, increasing attention has been paid to the development of biosensors for environmental diagnostics. The development of suitably robust biosensors, however, has been hindered by several problems associated with the properties of biological material: poor stability, poor performance in organic solvents, at low and high pHs and at high temperature; absence of enzymes or receptors that are able to recognise certain target analytes; problems with immobilisation of biomolecules; and poor compatibility with micromachining technology. The search for possible solutions to these problems has led to the development of synthetic analogues of natural receptors and antibodies. This keynote lecture will briefly review some of the biosensors and biomimetic sensors developed in our laboratories which can find application in a number of testing areas related to environmental monitoring. These include biosensors, immunosensors, electronic nose technology and molecularly imprinted polymer sensors for on-site testing for the detection of pollutants, toxins, GMOs and endocrine disrupters.

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A13.4
Microbial whole cell sensor systems for environmental monitoring

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At the heart of every biosensor is a biological entity, the purpose of which is to react with the target analyte(s) and generate a readily quantifiable signal. ''Traditional'' biosensors are based on the unique specificity of enzymes to their substrates, antibodies to antigens or that of nucleic acids to their complementary sequences. In recent years we have promoted the use of a different concept, that of whole cell biosensors. In such systems, an intact live cell, genetically engineered to respond in a quantifiable manner to changes in environmental conditions, serves as a combined sensing/reporting element. Using this approach we have constructed various microbial sensing systems for the environmental detection of toxicants, genotoxicants, oxidants and specific pollutant groups, as well as sensors of nutrients bioavailability.
In order to turn these cells into ''real'' biosensors, they need to be incorporated into a solid platform and coupled with a signal transduction apparatus. Several directions that were pursued to achieve this aim will be discussed, including encapsulation at the tips of optic fibers, embedding into sol–gel matrices and, most recently, integration into specialized biochips. The latter option is specifically oriented at the detection of toxicity in drinking water.

A13.5
Biosensors for marine applications

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Protection of the marine environment from negative anthropogenic impacts involves careful monitoring of a large range of different analytes and parameters. Because of the nature of the ''sample sea'' a varied range of strategies has to be employed and particular obstacles overcome. For example harsh working conditions, both for humans and sensors, high cost of research vessel time, inaccessible sampling sites, a complex and highly corrosive matrix, frequently very low analyte concentrations and aggressive biofouling can at times make the analytical task seem insurmountable. But the range of drivers for the development of biosensors for marine applications is equally impressive: euthrophication,, compliance monitoring, biodiversity issues and habitat protection (with the corresponding directives and legislation), and of course ecotoxicology. In addition to

issues surrounding pollution event or impact monitoring, sensors can be valuable tools aiding our fundamental understanding of ecosystem processes. Recording time series in situ, tracing profiles through sediments and resolving cycles and periodic events, as well as revealing the movements and potentially exposure of individual animals to toxic compounds.

Rapid progress is being made in two discrete areas: development of assays, sensors and diagnostic tools for ecotoxicology and development of strategies and platforms for continuous ecosystem monitoring, such as moorings, towed bodies and bottom landers. This paper aims to provide an overview of some of the current measurement strategies and biosensors available, under development or desirable for marine applications. Instrumentation requirements will be outlined in an attempt to set the scene for future generations of biosensors for marine applications.

A13.6
μMAC-ToxScreen: a novel automatic luminous bacteria-based early warning online monitor for water toxicity

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μMac-ToxScreen is an innovative automated online water quality monitoring system that uses luminescent bacteria biosensors to detect μg/L concentrations of toxic organic and inorganic chemical pollutants in surface or ground water, as well as raw and treated drinking water. The ToxScreen bioassay uses a renewable suspension of luminescent bacteria. When the bacteria are automatically mixed with a water sample, their light production, which is directly tied to cell respiration and other critical metabolic pathways, is decreased in proportion to the toxicity in the sample. The analytical part of the instrument is an automatic analyzer that uses a patented technology called Loop Flow Analysis (LFA) widely used in a variety of online chemical analyzers for water quality. At 14 day intervals, the instrument is re-supplied with a fresh inventory of liquid assay buffers and a freshly hydrated suspension of the freeze dried luminescent bacteria. Automatic safeguards have been engineered into the system to assure reagent and data quality and appropriate instrument functioning. The instrument is also equipped with auto calibration features to assure reliable instrument performance; microprocessor based system controls provide for data storage, data downloading, real time communication with a remote PC, and user adjustable alarm levels.

A13.7
Biosensor applications in toxicity determination of chemical industrial effluents

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In this study, whole cell biosensors were used to determine the toxicity of effluents from chemical industrial discharges as part of the technology demonstration project for rapid aquatic toxicity screening test methods. On eight occasions over a period of 13 months the toxicity of a total of 38 effluents from seven different sources was tested. Cellsense® is a rapid biosensor toxicity test enabling the concurrent monitoring up to 32 bacteria based biosensors electrodes during their exposure to environmental samples. Activated sludge consortium and *Escherichia coli* based biosensors were used in this test programme. EC_{50} (30min) were determined for each effluent and results were compared with the standard *Daphnia* and algae screening tests and other newly developed test methods. Seven test methods were also compared with regard to other technical aspects to determine the practicability and the costs of the assays, including – sample volume needed, test duration, type of test organisms, major equipment costs, test complexity, etc. The test duration was the same for the Daphtoxkit (*Daphnia*) and the Algaltoxkit (*Selanastrum capricornutum*) as for the standard Daphnia and algal tests (48 hours and 72 hours respectively), 24 hours for the Thamnotoxkit (F.W. crustacean *Thamnocephalus platyurus*), 12 to 20 hours for the Gentronic Cytotox (Green Fluorescent Protein expressing brewer's yeast) and 0.5 hours for the Cellsense. Cellsense was the most rapid assay employed, gave the highest sensitivity and good correlation to standard tests in the case of many of the samples tested. It also provided a most responsive screening test for decision making on effluent discharge.

A13.8
InLit-novel multiparameter fungal based biosensor

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Informative Light Technology (InLit) is a multiparameters toxicity bioassay based on cells expressing

recombinant aequorin gene. Aequorin is a Ca^{2+}-sensitive photoprotein, which emits blue light when binding to $[Ca^{2+}]_c$. Aqueous environmental samples can be readily applied to filamentous fungi growing in 96 well plates and the toxicity of the samples can be easily determined based on the profiles of $[Ca^{2+}]_c$ changes in response to addition of the toxicants. These profiles are called Ca^{2+} signatures and can be characterised by 15 parameters such as changes in amplitude, length of transients and final $[Ca^{2+}]_c$ resting levels. It has been determined that InLit technology can be also used for producing dose response curves, identification of toxicants mode-of-action and quality analysis of the mixtures of toxicants. A library of Ca^{2+} profiles has been created of known toxicants such as Cr^{6+}, Zn^{2+}, 3,5-dichlorophenol, pentachlorophenol, toluene, SDS. The fungus responded to heavy metals and toluene by a decrease in the $[Ca^{2+}]_c$ response to 5 mM external $CaCl_2$. When treated with chlorophenols and surfactants significant increase of the $[Ca^{2+}]_c$ resting levels was observed.

This study demonstrates that InLit based fungal biosensor may be used to determine the toxicity of a range of environmental pollutants to eukaryotic cells.

A13.9
New tools for quantifying gene responses in environmental toxicology

I. Weeks, J.S. Woodhead, C.A. Morris, R. Morgan and R.E. Thomas-Jones, (Molecular Light Technology Research Ltd, Cardiff, CF14 5DL, UK)

There is increasing interest in the use of gene transcription as a tool for the investigation of physiological or pathological processes. We have developed a system for the quantitative assessment of gene transcription based on a chemiluminescent hybridisation protection assay (HPA). In this method, an acridinium ester labelled oligonucleotide probe is reacted with samples containing target mRNA. Hybridisation serves to protect the label from hydrolytic degradation to a non-chemiluminescent derivative. A chemiluminescent reaction is initiated following a selection step. The intensity of light emission is dependent on the amount of target mRNA present in the sample. This technique is extremely specific and as little as 0.1 fmol of mRNA can be detected routinely, without the need for an amplification step. We have used the method to demonstrate increases of up to 1000-fold in the expression of vitellogenin and vitelline envelope protein genes by immature rainbow trout exposed to estradiol and ethinylestradiol. We have also succeeded in demonstrating increased expression of uterine C3 in rats exposed to diethylstilbestrol. This method thus provides an opportunity to investigate endocrine disruption in mammals. Further, we have developed a method for monitoring cytochrome P450 expression in vitro by measuring changes in the expression of CYP1A1 and 1A2 in the rat hepatoma cell line H411E following exposure to benzo(a)pyrene. In summary, we have shown that by virtue of its high specificity and sensitivity, HPA is a valuable tool for the identification and monitoring of responses to xenobiotics both in vivo and in vitro.

A13.10
The application of acute toxicity tests for the direct toxicity assessment of complex potentially toxic effluent discharges to the aquatic environment

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The combined use of biological effects measures (toxicity tests, bioassays, and biomarkers), substance-specific assessment and ecological community surveillance provides a powerful mechanism (the so-called ''triad approach'') for managing the ecological effects of potentially hazardous substances likely to enter or already present in the aquatic environment. For example, the OSPAR Joint Assessment and Monitoring Programme (JAMP) and the UK National Marine Monitoring Programme (NMMP) are founded on the ongoing development and implementation of such an integrated and holistic approach to environmental monitoring and assessment.

In recent years, the environmental regulators and industry in the UK have invested considerable resources in the development, validation and refinement of an infrastructure to support the application of Direct Toxicity Assessment (DTA) [– a stepwise, risk-based approach that uses standard acute toxicity tests, in conjunction with substance-specific assessment –] for the identification, assessment and control of potentially toxic discharges.

There is now broad agreement on how DTA should be applied in the UK and this is influencing the development of environmental regulation. although the UK DTA protocol currently applies solely to the assessment of acute toxicity, it provides a framework which, given modest R&D investment, could be extended to other hazardous properties such as chronic toxicity and endocrine disruption.

This paper outlines the development and application of DTA for the assessment and management of discharges

within the UK and pays particular attention to the lessons learned and the future research requirements.

A13.11
Bioassays: are they useful tools when implementing the habitats Directive in England and Wales?

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Implementation of the EU Habitats and Birds Directives in England and Wales requires that discharges subject to Environment Agency authorisations are reviewed to determine whether they could adversely affect European Sites. Authorised discharges that are, as a result of this risk assessment, found to significantly impact on the condition of these sites, either alone or in combination with other discharges, can be subject to amended conditions or revoked.

Current Environment Agency practice is to assess the risks to European Sites, or more specifically to the features and sub-features of interest that comprise them, on the basis of compliance with Environmental Quality Standards (EQSs) in the water column and, to a lesser extent, sediments. The limitations of this approach (e.g., a paucity of EQS values, or the potential for additive, synergistic or food-chain effects of contaminants) have prompted the Environment Agency, with the statutory conservation agencies, to appraise the use of bioassays within the review of consents procedure.

A series of standardised effluent, water column and sediment bioassays were combined with effluent plume modelling, analytical chemistry, benthic surveys, Toxicity Identification and Evaluation procedures, and bioaccumulation studies to assess the condition of two European sites: Seal Sands, Teesside and Southampton Water, Hampshire.

This paper presents the results of the appraisal and demonstrates how the data obtained from the Tees and Southampton Water studies has been used within the current Agency review of consents procedure. When applied appropriately, i.e. as part of a conceptual site-model, bioassays can provide information that is of additional value to the consents review procedure.

A13.12
Microbiotests: the new tool for cost-effective routine toxicity screening and biomonitoring

G. Persoone, (Laboratory for Environmental Toxicology, University of Ghent, Belgium)

Toxicity testing of chemicals and toxicity monitoring of contaminated waters or soils is expensive because of the necessity of year-round culturing and maintenance of live stocks of test species. During the last decades, alternative assays have been developed, which are independent of the former burden. Once such new approach is based on ''dormant, inactive, or immobilized'' stages of selected biota, which can easily be stored and from which live test organisms can be obtained ''anytime and anywhere'' to perform the assays. The miniaturisation of these assays in practical and low cost ''Toxkit microbiotests'', with various species representative of different trophic levels, opened the door for routine toxicity monitoring of contaminated aquatic and terrestrial sites. Extensive validation studies have been performed over the last few years in various laboratories in different countries, showing that the highly standardized Toxkit microbiotests are as sensitive as the ''conventional'' tests prescribed for testing in a regulatory framework. Toxkit bioassays are presently in current use in about 40 countries worldwide for a variety of applications such as toxicity ranking of pure chemicals, toxicity testing of surface and groundwaters, effluents, sediments, solid waste leachates and soils. As of to date more than 100.000 bioassays have already been performed worldwide with the culture/maintenance free low cost Toxkit microbiotests and more than 150 titles and abstracts of scientific papers can be found on the website www.microbiotests.be Examples of applications Toxkit microbiotests in different domains will be given during the presentation.

A13.13
Pragmatic low cost approach to the routine ecotoxicity testing of environmental samples

K. Wadhia and K. C. Thompson, (ALcontrol Laboratories, Rotherham S60 1BZ, UK)

The toxic impact of anthropogenic activity on the environment has in the past been predominantly defined in terms of chemical analysis. The use of biological effects to ascertain level of contamination represents a significant advance in direct toxicity assessment. Ecotoxicity

testing providing fit-for-purpose and robust methods for routine evaluations is of primary importance and consideration and a beneficial tool complementing chemical analysis. This paper describes routinely employed tests particularly pertinent to various industries. The tests include respiration inhibition, nitrification inhibition, use of dormant organism technology and a rapid bioluminescent test. The cost of making toxicity measurements has in recent times been dramatically reduced with the use of microscale organisms. A significant development in ecotoxicity testing has been the use of cryptobiotic stages of dormant biological material from which the test species can be hatched for performance of the bioassays. This has overcome the dependency on the maintenance of continuous cultures of live stocks. These new assays (named Toxkits) enable toxicity assessment of freshwater, marine and terrestrial environments and allow for cost-effective determinations with battery of different test species. The paper will describe the use of these tests for environmental ecotoxicity testing.

A13.14
Bioassay-directed analysis of environmental toxicants

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Monitoring programmes for contaminants require information on the types of hazardous substances that contaminate marine ecosystems. Existing approaches assess the risk posed by recognised contaminants with established ecotoxicological effects in order to prioritise those that need to be monitored. The process by which hazardous substances are prioritised is not flawless and quite often the concentrations of the targeted compounds analysed in monitoring programmes do not explain the ecotoxicological effects that are observed. Biological effects monitoring offers broad spectrum screening of environmental samples, however does not provide information on the types of compounds that are causing the observed effects. One approach that allows the hazardous substances that are having a demonstrably detrimental effect on marine ecosystems to be identified is bioassay-directed analysis (also referred to as toxicity identification evaluation; TIE). This paper provides examples of how bioassay-directed analysis can be integrated with a biological effects monitoring and how the data generated can be used to inform monitoring programmes. Examples include the identification of sediment bound mutagens in estuaries and endocrine disrupters in effluents and estuaries. The benefits of such an approach in focusing the resources available for monitoring are also discussed.

A13.15
Combined use of a cyclodextrin extraction technique and *Daphnia magna* for the assessment of mono- and polycyclic aromatic hydrocarbons bioavailability in dissimilar contaminated soils

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For a pragmatic assessment of soil contamination as well as for the prediction of contaminated soil bioremediation endpoints, it is desirable to be capable of quantifying not only total concentrations of pollutants but also the bioavailable fraction of contaminants in soils. A chemical extraction technique using an aqueous solution of hydroxypropyl-β-cyclodextrin (HPCD) has, so far, been successfully tested as a mean to measure the microbial bioavailable fraction of single contaminants in relatively clean soils. The work presented here relates to the use of the HPCD solutions to simultaneously assess the bioavailable fractions of PAHs and phenolic compounds in 2 mm-sieved contaminated soils from MGP sites. The microbial bioavailability of phenanthrene, pyrene, benzo[a]pyrene and *p*-cresol was assessed using microbial biodegradation assays with catabolically active degraders for these four compounds. Results obtained for degradation were compared to compound extractability using the HPCD extraction and an aqueous leach test. The three soils were then lab-spiked with a mixture of deuterated PAHs (namely phenanthrene, pyrene, and benzo[a]pyrene) and after equilibration the procedure described above was repeated to expand the study to the assessment of freshly added contaminants. Toxicity of the resultant HPCD extracts was assessed using *Daphnia magna*, for comparison with the other results. In order to investigate possible synergistic or antagonistic effects of the presence of HPCD molecules, the toxicity of aqueous extracts containing a range of concentrations of a single PAH with and without HPCD was also assessed using *Daphnia magna*.

A13.16
Reproductive effects of endocrine active chemicals in pair-breeding fathead minnows

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Natural and synthetic chemicals can interfere with the endocrine system and disrupt normal reproductive function via different mechanisms. Available testing methods for endocrine active chemicals (EACs) focus on the detection of chemicals that act via the oestrogenic pathway. More integrated testing methods are required to understand the wider effects of EACs. In this project a reproductive performance assay was assessed for its ability to identify chemicals with both oestrogenic and anti-androgenic mechanisms of action. Pair-breeding fathead minnow were exposed, for a period of 3 weeks, to (a) the natural oestrogen, oestrone (32 to 1000 ng/L) and (b) an anti-androgen, linuron (22 to 2200 μg/L). Exposure to oestrone reduced survival of males, and inhibited growth of both males and females at concentrations above 320 ng/L, but a reduction in fecundity was only observed at 1000 ng/L ($P < 0.05$). No effects were observed on gonadal growth or secondary sexual characteristics. Increases in plasma vitellogenin concentrations occurred in both the males (LOEC 32 ng/L) and females (LOEC 320 ng/L). Linuron did not affect survival, growth, gonad weight, or plasma vitellogenin concentrations, but reduced both prominence of male secondary sex characteristics (LOEC 220 μg/L) and egg production (LOEC 1000 μg/L; $P < 0.05$). The fathead minnow pair-breeding assay is thus an effective test for detecting oestrogens and anti-androgens. The biomarkers incorporated can inform on the mechanisms of action. Work is ongoing to assess the effects of EACs with other modes of action.

A13.17
The application of genomic technologies to environmental monitoring

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Genomic technologies using microarrays can detect thousands of gene transcripts in a single biological sample simultaneously and is a very powerful approach to identifying altered gene expression in disease states or in response to a toxic agent. Toxicogenomic approaches are being developed for environmental studies but are currently impeded by the limited availability of genomic resources for environmentally relevant species. We have adopted a focused strategy to generate genomic resources that produces cDNA libraries enriched for pollutant-responsive gene transcripts. This is achieved by Suppression Subtractive Hybridisation (SSH) and the resulting probes are used on macroarrays for the analysis of biological samples. SSH was used to generate cDNA libraries from liver of ethynyloestradiol (EE2)-treated male plaice (*Pleuronectes platessa*) and from digestive gland of benzo[a]pyrene (BaP)-treated common mussel (*Mytilus edulis*). Screening and subsequent characterisation of selected clones identified about 100 non-redundant expression sequence tags (ESTs) from both the plaice and mussel libraries. The plaice ESTs included many vitellogenin cDNAs from at least 3 genes and zona radiata proteins (3 genes), liver aspartic protease (LAP) and over 20 cryptic clones. The mussel library included cryptic clones, general stress proteins (e.g. HSP70, defensin), lysosomal proteins (ganglioside GM2 activator, proteolytic enzymes) and proteins involved in: oxidative stress (metallothionein, methyltransferase, superoxide dismutase); energy metabolism (cytochrome c oxidase, isocitrate dehydrogenase, NADH dehydrogenase) and cell signalling and differentiation (Notch, EGF).Unique ESTs were used to produce species-specific macroarrays, which were validated by analysis of mRNAs from plaice and mussel exposed to EE2 and BaP respectively. Results are presented from the use of the mussel macroarray to investigate potentially contaminated environmental sites.

A13.18
The use of biomarkers for the assessment of chronic pollution

R.D. Handy, (School of Biological Sciences, University of Plymouth, UK)

There is now a wealth of information on biomarkers, but they are not routinely used for regulatory purposes, even though the potential benefits of biomarkers are clear. Biomarkers have not been applied to chronic pollution problems, partly because they have been misunderstood in the context of dose-effect and spatial/temporal variations in biomarker responses. Here, we review the geochemical and biotic factors causing temporal and spatial variability in biomarker responses. The variation can be minimised by appropriate study site selection, experimental replication, multi-variate epidemiological approaches, normalised controls, and temporal calibration of responses; so that the regulatory use of biomarkers for biomonitoring and tracking pollution events, including chronic or multiple exposures to complex

mixtures is possible. We propose and define the characteristics of biomarkers of chronic exposure or effect, which must measure changes in pollution/effect against long-term changes in other general stresses (disease, nutrition, environmental quality), relate to cumulative injury, and remain responsive over months or years. Neuro-endocrine, immunological, and histological biomarkers are suggested for chronic pollution. We propose a regulatory framework for biomarkers based on a weight of evidence approach.

A13.19
Defining operational frameworks for the use of biomarkers in regulatory environmental protection

R. Owen, (Ecosystems and Human Health Group, Environment Agency)

If wisely and correctly used, biomarkers show great promise in terms of identifying whether ecological harm is currently happening (i.e. diagnosis) or will occur (prognosis) as a result of human activity. Whilst there have been considerable advances in development of biomarkers (i.e. the tools), a consensus on the framework for their applied use within a regulatory context is yet to be reached. Establishing this framework will define the tools required and the future research agenda for their development, calibration, accreditation and implementation.
We open a discussion that we hope will lead to establishing such a consensus. We suggest a bilateral approach for use of biomarkers within a regulatory context. In a diagnostic capacity we suggest there is an immediate use for biomarkers in establishing causality between sources of complex pollutants (e.g. industrial effluents, sewage treatment plants) and impact in receiving waters – i.e. an evidence-based approach. Used as an initial screening this could provide evidence to justify management action e.g. recommending further (and more costly) detailed toxicity identification and reduction plans. In prognostic capacity biomarkers may also act as early warning indicators of unacceptable ecosystem health decline, justifying early intervention prior to this. Molecular epidemiology approaches used in human health risk prediction may provide a framework for this, with the understanding that in ecotoxicology biomarkers may need to be validated beyond the organism level (e.g. ecosystem responses). Greater challenges are to establish an operational definition of ecological harm, what constitutes unacceptable harm and how biomarker responses relate to envelopes of acceptability.

A13.20
The impact of post-genomic science on biomarker identification and validation for environmental biomonitoring: an applied perspective

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Rapid progress in the field of genomics (the study of how an individual's entire genetic make-up, the genome, translates into biological functions) is now providing biomarkers that increasingly support our understanding of how chemicals impact on human and ecosystem health. Arguably, if scientific efforts in the 20th century have focussed on testing key chemicals impacting ecosystem health, then the ecotoxicology challenge for the 21st century is to understand the mechanistic basis of ecotoxicity such that environmental (ecological) risk assessment (ERA) becomes more cost-effective. In the human context, '*toxicogenomics*' is routinely used to describe the study of gene expression in adaptive responses to toxic exposures. Given the parallel implications for ERA, we propose the term '*ecotoxicogenomics*' to describe the integration of post-genomic science into ecotoxicology. Both toxicogenomics and ecotoxicogenomics may provide us with a better mechanistic understanding of (eco)toxicology such that we can identify relevant biomarkers. However, both disciplines face a possibly greater challenge, namely distinguishing between molecular adaptation (*biomarker signals*) versus population relevant adverse effects based on endpoints such as survival, development, growth and reproduction (*adverse effects for supporting ERA*). This presentation will draw upon the output of the International Life Sciences Institute (ILSI) toxicogenomics study to demonstrate how relevant, predictive and reproducible biomarkers can be identified from standard and non-standard (eco)toxicological studies. The importance of the single molecular biomarker approach versus a gene cascade approach in order to guide subsequent population level studies (which directly underpin environmental risk assessment) will be emphasised.

A13.21

Integrating ecotoxicology and analytical chemistry to assess water quality status for river basin management

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The EC Water Framework Directive aims to maintain and improve the quality of aquatic ecosystems in the EU by using an integrated approach to the protection, improvement and sustainable use of the water environment. Nitrate and nitrite concentrations in natural waters rarely exceed the maximum admissible levels set by the EU for surface waters intended for abstraction for drinking water (11.3 mg L^{-1} NO_3-N) and for the protection of course freshwater fish (9.0 μg L^{-1} NO_2-N) respectively. However, in cases of extreme pollution, levels may be as high as 1 mg L^{-1} NO_2-N such as in waters strongly influenced by industrial effluent, or 200 mg L^{-1} NO_3-N in sewage polluted waters. Rapid (potentially field based) ecotoxicological tests has been used in this study to determine the effect of sub-lethal levels of nutrients on the overall health of a selected freshwater mollusc, *Anodonta cygnea*. Preliminary toxicity studies indicate that 96 hour EC_{50} values are in excess of 2 g L^{-1} nitrite and 10 g L^{-1} nitrate for this species; and the 24 and 36 hour LC_{50} values for nitrite were 2 and 5 g L^{-1}. Spectrophotometric determination of engulfed red dye particles in haemocytes (phagocytosis assay) or the uptake of the dye by lysosomes (neutral red retention assay) was used to monitor immune function and cell viability of Anodonta cygnea when exposed to sublethal concentrations of nitrite. Cardiac activity was also be monitored during these exposures, using a computer-aided physiological monitoring system (CAPMON) based on photodiode detection. Together these biomarkers allow an assessment of cell and organismal health. This integrated approach will allow the assessment of surface water quality based on the chemical and biological objectives of the Water Framework Directive.

A13.22

Low cost automatic activity monitoring of the amphipod *Gammarus pulex*, and its role in ecotoxicity testing

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Gammarid amphipods are important components of the aquatic ecosystem. They are omnivorous, utilising a wide variety of food sources including plants, dead animals and invertebrates. Gammarids in turn form a significant part of fish diet. The amphipod *Gammarus pulex* is widely distributed in standing and running fresh water habitats in Britain. It has been extensively used for ecotoxicology studies ranging from LC_{50} tests to biomonitoring applications. Current non lethal ecotoxicology tests on this organism are either laborious to perform and/or require relatively expensive equipment. We report the development of a new low cost infra red actograph system that measures relative activity of gammarids. Preliminary tests of this system demonstrate that it can readily detect very low concentrations of heavy metals. The simple design, cheap components, and high sensitivity of the equipment renders this method a useful addition to the repertoire of ecotoxicology tests.

A13.23

An evaluation of the relative sensitivity of cyto- and genotoxic biomarkers in two marine bivalve mollusc species

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In biomonitoring studies, the marine bivalve *Mytilus* sp. has been widely used as a sentinel species. For comparison, the common cockle *Cerastoderma edule* was used in this study as an alternative species for detecting biological effects of contaminants in marine and estuarine environments. In vitro validation studies were carried out on haemocytes that were collected from the posterior adductor muscle of adult *Mytilus edulis* and *Cerastoderma edule*. The samples from each species were pooled and sub-samples exposed to a range of concentrations of hydrogen peroxide (H_2O_2), a known oxidant. The level of DNA damage induced was measured with the 'comet assay', which detected a dose-dependent increase. Following these studies, haemocytes were collected from indigenous populations (in vivo) of the two species, located at six sites along the Tamar estuary (SW Devon). The 'comet' and 'neutral red lysosomal retention' assays were used in an attempt to determine dif-

ferences in levels of genotoxic and cytotoxic impacts respectively, between different populations. A pollution gradient was detected with increasing levels of DNA damage being measured in samples from animals collected from sites located upstream, away from the mouth of the estuary. The level of DNA damage correlated with in vivo cytotoxicity measurements, in addition to heavy metal concentrations quantified in the sediments and soft tissues of the biological samples. Results from the *Cerastoderma edule* indicated a stronger correlation between biological effects and levels of heavy metals measured in the sediments compared with *Mytilus edulis*, probably due to the different habitats of the two species.

A13.24
The development of a cell culture system to determine bioavailable metal in natural water samples using molecular endpoints

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A cell culture system has been developed to investigate bioreactivity of metals from naturally occurring waters. Rainbow trout primary gill cells are grown on cell culture inserts, allowing apical cell culture media to be replaced by water samples, while the basolateral side of the cells are maintained in media. When determining metal bioavailability and toxicity it is important to consider the form of the metal. This system is ideal as gill cells can be directly exposed to complex water samples in vitro without compounding agents normally present in cell culture. The bioreactive fraction of metal in the water sample can be detected using the cells naturally occurring metal monitor, Metal Transcription Factor 1 (MTF1), which acts upon Metal Response Elements (MRE's) in the promoter region of specific genes causing up-regulation. The metal responsive genes used are metallothioneins (MTA and MTB), which are cysteine-rich low MW proteins that bind transitional metals, and Zinc Transporter 1 (ZnT1), a basolateral zinc exporter. These genes have multiple MRE copies and have been shown to respond with a significant induction to a variety of metals including copper, silver, zinc and cadmium over a 24–48 h exposure period. This response is dose dependent and relatively specific to metals, identifying this system as a potential biomonitoring tool. Furthermore metal competition studies using a variety of biological ligands (e.g. Ca^{2+}, Na^{+}, DOC, pH and Cl^{-}) have been carried out showing a comparative reduction in metal responsive gene transcription levels, reflecting the bioavailable metal.

A13.25
Endocrine disruption in large pelagic predators: the role of biomarkers and immunochemistry in rapid assessment monitoring

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Vitellogenin has been considers as a biomarker for the presence of endocrine disruption in a number of freshwater species and recent literature reports have implicated this substance in large pelagic predators. We report levels found in both tissues and plasma of two of the large pelagic predators, the swordfish *xiphias gladius* and the Bluefin tuna, thunnus *thynnus* and comment on the levels found in male fishes and there use as biomarkers. In concomitant histochemical studies using cytochrome P4501A (CYP1A) monoxygenase, vitellogenin (Vtg) and *zona radiata* proteins (Zrp), which are frequently used as biomarkers of fish exposure to organic contaminants, swordfish liver sections obtained from the Mediterranean Sea, the South African coasts (South Atlantic and South Western Indian Oceans) and the central North Pacific Ocean were immunostained with antisera against CYP1A, ZRP, and Vtg. CYP1A induction was found in hepatocytes, epithelium of the biliary ductus and the endothelium of large blood vessels of fish from the Mediterranean Sea and South African waters, but not from the Pacific Ocean. These results confirm previous findings about the potential exposure of Mediterranean swordfish to endocrine disrupting chemicals and raise questions concerning the possible presence of xenobiotic contaminants off the southern coasts of South Africa in both the south Atlantic and southwestern Indian oceans. The alternative roles of Vtg and Zrp determinations in plasma and muscle compared to immunochemistry as rapid testing agents are discussed.
Financial support provided by EU grant CFP - BFTMED - 97/0029; SIDS QLK5-CT1999.01567; REPRODOTT QLRT-2001-01355

A13.26
Can Microtox test results be used to predict toxic responses in the marine diatom *Skeletonema costatum*?

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This study investigated the use of Microtox rapid screening test results to predict toxic effects in the marine diatom *Skeletonema costatum*. The Microtox and *S. costatum* toxicity data used in the analysis were collected from the open literature. Appropriate 30 minute IC50s for Microtox and EC50s for biomass and population growth for *S. costatum* were located for 21 chemicals covering a range of toxic modes of action. Results of regression analysis with the 21 chemicals demonstrated a strong relationship between the responses in the two assays. The relationship was strongest for chemicals of a narcotic and polar narcotic mode of action. However, predictions of *S. costatum* toxicity from Microtox data are associated with high variability. In practice, the levels of uncertainty are likely to be too great for accurate ranking of chemicals with respect to algal toxicity and is certainly too great to predict ''absolute'' toxicities to this species.

A13.27
Production of metal-binding peptides by phytoplankton in metal contaminated estuarine waters

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The Fal Estuary (Southwest England) is subject to metal inputs from acid mine run-off, urban and agricultural waste discharges, and leaching of Cu from antifouling paints from boats. intracellular metal-binding peptides such as phytochelatins and glutathione are produced by a number of organisms as a metal detoxification mechanism and regulation of intracellular metal concentrations. Dissolved organic ligands in natural waters can also bind metals and thus ameliorate metal toxicity by decreasing the concentrations of bioavailable metals. In this work we investigated the production of phytochelatins and glutathione by phytoplankton and its relation with Cu complexation in the Fal Estuary. The highest phytochelatin concentrations were found in Restronguet Creek, where dissolved organic ligands can often be found saturated by Cu. Glutathione was found at constant concentrations throughout the estuary. Phytochelatin concentrations were higher in the first campaign (2002), despite the excess of dissolved organic ligands in relation to the total dissolved Cu. Iin the second campaign (2003), the concentrations of dissolved organic ligands were close to the concentrations of total dissolved Cu and phytochelatins were at concentrations close to (or below) detection limit. Cu-organic complexes presented high conditional stability constants in the range for the strong ligands. Concentrations of ionic Cu, the most toxic species, spanned from 10^{-7} to 10^{-15} M in the estuary. Weaker organic ligands should also be important to buffering the heavily metal contaminated waters, and minimise the adverse effects of metals.

A13.28
Application of biomarkers and biological profiling approaches for measuring harm in soil ecosystems

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Post-genomic and profiling technologies have the potential to identify molecular levels responses that can then be employed in a regulatory setting for the sensitive detection of exposure to, and effect from, specific environmental contaminants. The movement of molecular level assays from academic toys to a legislative tools will, however, only ever be accomplish if efforts are made to establish not only their inherent potential, but also their fundamental limitations. This poster will give a brief overview of our recent work to establish the use of a series of molecular genetic approaches for assessing the exposure of soil invertebrates to metals, halogenated aromatic compounds and potential endocrine active chemicals. Focus will be on studies recently completed in which we have measured of the expression of genes encoding an invertebrate metallothionein and an invertebrate hormone and also profiled global small metabolite responses to chemical exposure. In this work we have 1) assessed the significance of molecular changes with respect to whole organisms biology, and population and community status; 2) assessed the comparative sensitivity of molecular genetic and ecological responses; 3) established the effects of environmental conditions including soil parameters and climate on measured molecular responses; 4) established invertebrate metabo(l/n)omics as a tool for identifying chemicals with potentially different modes of action.

A13.29
Abstract not supplied

A13.30
Toxicity Testing of Linear Alkylbenzene Cable Oil using *Caenorhabditis Elegans* in Soil

S. Johnson*, M. Castan†, L. Proudfoot^{+} and N. Christofi (Pollution Research Unit and $^{+}$Biomedical Research Group, Napier University, Edinburgh, EH5 10DT, UK.www.napier.ac.uk; *now at University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4AW, UK; †Ecole Polytechnique Universitaire de Lille, Departement IAA, Boulevard Langevin – F, 59655 Villeneuve D'ascq Cedex, France)

Linear alkybenzene (LAB) is an LNAPL used as a precursor in the manufacture of linear alkylbenzene sulphonate detergents, from where it makes its way into the aquatic environment. LAB is classified N; R50 Very toxic to aquatic organisms. In addition, LAB is used as an insulating oil in underground electricity transmission cables. Failure of a cable may result in contamination of soil with cable oil. To date, no data have been published on the toxicity of LAB to soil organisms.
Nematodes occupy a central position in soil ecosystems, and since they live in interstitial water they are exposed to dissolved contaminants. They are therefore good candidates for ecotoxicity studies and have been used in the assessment of environmental contaminants. *Caenorhabditis elegans* is a non-parasitic soil nematode that is already well known because of its use in developmental studies. Its small size, hermaphroditism, short life cycle and the occurrence of a stress-tolerant ''dauer'' larval stage means that it is easy to raise large numbers of genetically similar, age-synchronised adults in the laboratory. A 24-h acute exposure to heavy metals has been shown to be equivalent to a 14-d earthworm assay and an ASTM method (E2172-01) for acute toxicity of substances in soil has been published.
Populations of *C. elegans* were exposed to several concentrations of LAB in natural soil. Nematodes were recovered using differential density centrifugation in colloidal silica. Mortality was determined after 24 h and the LC_{50} was calculated by several methods. Trimmed Spearman-Karber analysis yielded the most conservative estimate at 0.52% w/w dry soil, as well as the narrowest 95% confidence interval, with upper and lower confidence limits of 0.405% and 0.68%, respectively (n = 360). This figure is higher than published toxicity to aquatic organisms but LAB adsorbs to clay and humic material in soil so the availability to soil organisms will be rather lower than the absolute amount.

A13.31
Microbial biosensor for detection of low molecular weight PAHs

S. O'Neill and S. Ripp (FRS Marine Laboratory, Aberdeen, UK and University of Tennessee, USA)

Oil exploration, production and transportation within the North Sea have the potential to impact both on the environment and the fishing industry through hydrocarbon contamination. Previous pollution incidents have released oil containing a large percentage [>80% of total polycyclic aromatic hydrocarbons (PAHs)] of low molecular weight (2- and 3-ring) PAHs. Rapid detection of such contamination is the key to effective incident management. Fisheries Research Services Marine Laboratory (FRS ML) is investigating the potential of a microbial biosensor as a technique for use in environmental emergencies to detect low molecular weight PAHs.
Pseudomonas fluorescens HK44 contains a catabolic plasmid (pUTK21) mutagenised by transposon insertion of *lux* genes (King et al. 1990). The inclusion of the lux genes enables the microbe to bioluminesce as it degrades specific PAHs including the 2-ring compound, naphthalene. Light intensity can be measured and correlated to the relevant PAH concentrations and standard curves produced. In vitro studies at the FRS ML have demonstrated that HK44 can be used to identify naphthalene contaminated marine sediments (O'Neill et al. 2003) and fish muscle contaminated with low molecular weight PAHs.
HK44 cells have been incorporated into a fibre optic biosensor with a multiplex light detection system enabling direct detection of vapour phase naphthalene in contaminated soil (Ripp et al 2000). Successful field trials on contaminated land in the USA have been carried out.
The goal at FRS ML is to have a biosensor that can be taken on board research vessels enabling environmental monitoring at sea, thereby enhancing FRS ML's rapid response capability during major pollution incidents.
References:
King et al. 1990. Science 249:778–781.
O'Neill et al. 2003. FRS Marine Laboratory, Aberdeen, Collaborative Report No 05/03
Ripp et al. 2000. Environ. Sci. Technol. 34(5):846–853.

A13.32
Detecting hydrocarbon taint in fish

S. O'Neill[a], A. Craig[a] and S.Ripp[b], ([a]FRS Marine Laboratory, Aberdeen, UK; [b]University of Tennessee, USA)

Fisheries Research Services Marine Laboratory is accredited under the international standard ISO 17025

for assessing the presence of petrogenic taint in fish. The trained panel members use taste and smell to identify taint in fisheries products following marine oil spills.
This study examined the use of a genetically modified bacteria to indicate the presence of low molecular weight polycyclic aromatic hydrocarbons (PAHs) which are known to give rise to petrogenic taint. *Pseudomonas fluorescens* HK44 contains a catabolic plasmid (pUTK21) mutagenised by transposon insertion of *lux* genes (King et al. 1990). The microbe bio-luminesces as it degrades specific PAHs, including naphthalene.
Rainbow trout were exposed to water contaminated by Forties Crude Oil for periods up to 4 hours. The muscle tissue was assessed by the sensory panel and analysed for PAH using gas chromatography with mass selective detection (GC-MSD) (August 2003). Tissue samples were stored at −20 °C prior to being tested using HK44 (December 2003).
Total PAH concentrations in the muscle of treated fish (time series) were 212, 447, 584, 1780, 2072 and 2259 ng PAH/g, and 2 ng PAH/g in control.The sensory panel identified taint in all except the control sample and the 212 ng PAH/g fish.
Fish and HK44 (n = 4) were incubated for 7 hours 42 minutes at 30°C. HK44 luminescence readings distinguished the lowest dosing level of contamination (212 ng PAH/g) from the 2ng PAH/g control. At PAH concentrations >1700 ng PAH/g, luminescence readings decreased indicating a possible matrix effect. HK44 warrants further investigation as a potential method for identifying petrogenic, taint-inducing, hydrocarbons in fish.
Reference : King et al. 1990. Science 249:778–781.

A13.33
Responses of Free and PVA-Immobilised Genotoxicity Sensing Bacteria

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The efficiency of whole cell microbial biosensors is dependent on response time and sensitivity to a range of toxicants. Bacteria marked with luminescence genes (e.g. *lux, luc*) fused with promoter sequences of specific genes (responsive to DNA damage, oxidative stress, nutrient deficiency etc) have been used as reporter tools for gene expression. Such bacteria are often required to be incorporated/immobilised onto chips or optical fibres to report the gene activity (to permit/facilitate the transduction of the light signal to luminometry equipment). In the present study, *Salmonella typhimurium* harbouring a *recA*::*luxCDABE* fusion on a multicopy plasmid was used to assess the genotoxicity of various chemicals/pharmaceuticals. The organism was immobilised in pol yvinyl alcohol and its response to genotoxicity was tested following storage at −20°C for periods up to two months. The responses were compared to those of freshly prepared free-living organisms. It was found that gene induction following chemical challenge of immobilised bacteria required higher toxicant concentration indicating and that there was a decrease in sensitivity to genotoxic substances with time. Factors affecting the sensitivity of stored bacteria are discussed.

A13.34
Use of image analysis to determine endpoints in the oyster embryo larval development test

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The oyster-embryo larval development test (OEL) has been used for a number of years for direct toxicity evaluations of effluents, and in monitoring the marine environment. The conventional method of assessing larval development, involving the visual assessment of normal to abnormal ratios, is inevitably subjective and relies on an accurate definition of what constitutes abnormality. Imaging systems have now been developed which are capable of detecting and counting normal ('D' shaped) embryos based on a series of defining measurements. Such technology also allows the measurement of additional sub-lethal endpoints such as larval growth without altering the test methodology. The Environment Agency and CEFAS have progressed this application of the technology, and addressed a series of technical issues that have enabled the development of a robust, consistent technique for analysing oyster larvae. Recent work has focussed on the validation of the methodology for use within routine test programmes. Oyster embryos exposed to zinc as a reference toxicant, and to samples of industrial effluents and estuarine receiving waters, have been analysed using both conventional and imaging methodologies. No significant differences have been found between point estimate or Percentage Net Response (PNR) values derived using the imaging system and those generated manually, when embryos were exposed to zinc. This indicates that the imaging end point can be used to assess effluent and receiving water samples, and this is currently being validated.

A13.35
Linking biomarkers to whole organism and population effects of toxins: using *C. elegans* as a model system

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Biomarkers appear to be an attractive way of assessing whether organisms collected from the field have been subject to pollution stress. We often know that particular biomarkers are more sensitive than acute toxicity testing, but much less is known about the relative sensitivity of biomarkers and chronic toxicity. Does a biomarker response indicate that ecological damage is occurring? Or do biomarkers simply measure normal adaptive responses to stress? For example, a positive response on a ''lie-detector'' test indicates that a human is stressed, but not that they are in immediate danger of dying or of producing a reduced number of offspring relative to unstressed individuals. We have exposed *C. elegans* to metals and hydrocarbons and compared dose-response relationships for acute and chronic toxicity endpoints with those for a number of biomarkers, including RNA/DNA ratios and expression of metallothionein (MTL) and heat shock protein (HSP) genes. Biomarker responses can often be detected at concentrations at or below the chronic toxicity threshold. However, peak responses may occur only at concentrations that are close to those that are acutely toxic. The relationship between chronic toxicity and biomarker response varies markedly between toxins, and biomarker response can be markedly altered by nutritional status and temperature. So biomarkers are potentially sensitive indicators of stress, but we are still some way from being able to predict ecosystem effects of pollutants from data on biomarkers alone.

A13.36
Abstract not supplied

A13.37
The application of an effects-based assessment to study non-point source pollution in agricultural regions

M.A. Gray, S.M. Brasfield, and K.R. Munkittrick, (Dept of Biology and Canadian Rivers Institute University of New Brunswick, Saint John, NB, E2L 4L5, Canada)

Recently in Atlantic Canada, there has been increased concern associated with potato farming as a result of an increase in the frequency and magnitude of fish kills downstream of cultivation activities following major storm events. Over a period of four years, we have monitored the population structure and physiological performance of slimy sculpin (*Cottus cognatus*) in an intensive potato cultivation region of northwestern New Brunswick. Rather than focus on particular agricultural stressors, an effects-based assessment of the fish in the system was conducted to determine whether there were observable and consistent responses of sculpin in the agricultural region. We found that the local population structure at agricultural sites consisted of fewer young-of-the-year (YOY) sculpin in two of four years. In comparison with forested reaches, adult sculpin were larger, but with smaller gonads, and females had smaller livers, and fewer and smaller eggs. We have found a significant correlation between improved reproductive performance (i.e. numbers of YOY sculpin) with decreased rainfall, evident in agricultural areas but not at reference sites. The effects-based approach successfully demonstrated biological impacts on sculpin temporally and spatially and therefore the species' potential for studying non-point source impacts in environmental assessments. Follow-up studies are attempting to separate the roles that nutrient and chemical additions, sedimentation, habitat loss and changes in temperature and flow play in the biological responses observed.

A13.38
Stereological and GFP-transgenic approaches to establishing TCDD toxicity in zebrafish

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Persistent ecotoxicants such as dioxin and PCBs are thought to pose one of the greatest threats to public and ecological health in the industrial world. They cause a range of macroscopic malformations during the early sensitive stages of vertebrate development, particularly to the craniofacial apparatus, and to cardiovascular and neural systems. We have investigated the underlying mechanisms of environmentally-relevant exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by taking advantage of the technical conveniences of embryonic and larval zebrafish (*Danio rerio*). Firstly, quantitative stereological techniques have been used to measure cell number and tissue volume of the brain. We show that TCDD substantially reduces the capacity for embryonic brain development, causing a 30% reduction in total neuronal number and tissue volume in the 168 h larval brain. Second, transgenic GFP technology has been used to assess the in situ effects of TCDD exposure on expression patterns of candidate genes, namely zebrafish lines expressing GFP under the control of promoters for key developmentally regulated genes, *neurogenin* and

sonic hedgehog. Exposure at environmentally relevant doses caused substantially decreased and disrupted expression of both genes. This disruption of neuronal gene expression during early development of the neural system provides the basis for understanding the neurotoxic effects of these potent compounds. Both techniques demonstrate effects at both microscopic and molecular levels over the range of doses that cause malformation. They offer clear conveniences when applied quantitatively.

A13.39
Bio-analytical tools; YES, YAS and DR-CALUX assays and there use in R & D and monitoring in the aquatic environment

J. Thain and M. Hurst, (CEFAS, Burnham Laboratory, Burnham on Crouch, Essex. CM0 8HA, UK)

In vitro recombinant receptor-reporter assays are increasingly being used as bio-analytical screening tools for chemicals, effluents and environmental samples. The assays are cost effective because of their small sample size and high throughput. This paper describes the methodology of the screen for arylhydrocarbon (Dioxin Responsive Assay; DR-CALUX), oestrogenic (Yeast Estrogen Screen; YES) and androgenic (Yeast Androgen Screen; YAS) receptor-based activity. The assays allow the quantification of activity and produce activities in equivalent units for a relevant standard. During the past three years CEFAS have applied all three screens on water, effluent and sediment samples. Examples of data shown will include: Determination of dioxin-like activity in UK estuarine sediments, both total and cleaned up extracts. The concentration of the most stable dioxin-like compounds in the cleaned-up sediment extracts was between 1.0 to 106 pg TEQ_{CALUX} g^{-1} dry wt. in samples collected. The majority of sediments contained levels of dioxin-like compounds that were above concentrations that are thought to cause environmental harm. The CALUX bio-analytical approach showed some disparity with the traditional chemo-analytical approach. The reason for these differences have potentially been identified. Five produced water samples collected from oil production platforms in the British and Norwegian sectors of the North Sea were assayed using YES and YAS screens. Produced water samples were extracted in situ on the production platforms using large volume solid phase extraction. All five extracts tested positive for the presence of ER agonists (3–28 ng E2 L^{-1}), whilst no AR agonist activity could be detected. Further examples will be provided.

A13.40
The role of biomarkers in the UK National Marine Monitoring Programme

J. Thain, S Feist, B. Lyons and R. Reynolds, (CEFAS Laboratories), M. Gubbins and A. MacIntosh, (FRS, Aberdeen), and S. George, (University of Stirling, Stirling, UK)

The UK National Marine Monitoring Programme (NMMP) seeks to integrate national and international monitoring programmes across UK agencies and provide high quality marine monitoring data sets (e.g. for EC Directives and OSPAR). Biological effects techniques have been increasingly used in the programme and follow those required by the OSPAR Joint Assessment Monitoring Programme (JAMP). In 2003 a programme of flounder sampling took place in UK estuaries; Belfast, Clyde, Forth, Tyne, Alde, Thames and Southampton Water. The following JAMP biological effects techniques were used; EROD, bile metabolites, DNA-adducts, liver histopathology, metallothionein and external fish diseases. The methodology and results for each technique is described. Clear distinctions between estuaries were evident for each technique, however anticipated correlation between parameters was not so apparent e.g. high presence of DNA adduct did not show elevated bile metabolite or increased histopathological changes in fish of the same origin. The results are discussed in relation to contaminant concentrations within the estuaries. Mussels (*Mytilus edulis*) were sampled for metallothionein analysis in five estuaries, the Clyde, Tees, Thames, Southampton Water and Mersey. Metallothionein concentrations were 2- to 3-fold higher in mussels from the Clyde and Thames and slightly less than 2-fold higher in the Southampton population than those from the Tees and Mersey. Interestingly it appears that mussel metallothionein results do not follow those of flounder livers in the same estuaries.

A13.41
No Abstract allocated

A13.42
Application of biosensors to assess environmental contamination and remediation strategies

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A historically contaminated site with elevated levels of chlorinated solvents had been characterised using detailed chemical analysis. The chemical analysis in iso-

lation did not aid the site managers in terms of a suitable remediation strategy nor of the impact of these pollutants on environmental receptors.
Microbial *lux*-marked biosensors were used to identify areas where the pollutants were biologically available. A set of metabolic and catabolic biosensors allowed assessment of the general toxicity and quality of the present pollutants. The metabolic biosensor used was *Pseudomonas fluorescens* (a common soil bacterium). The catabolic biosensors were *Pseudomonas putida* TVA8, which is induced in the presence of aromatics and a series of chlorinated solvents and *Escherichia coli* DH5alpha, which responds to alkanes.
Furthermore, a series of pre-treatments was applied to assess the suitability of potential remediation strategies. These consisted of purging and charcoal treatment to remove volatiles only and total organic carbon, respectively.
The results support the suitability of using biosensors for the assessment of environmental toxicity. It is shown that it is likely that the introduction of an effective sparging technology will significantly reduce the levels of contamination associated with the site. Further refinement could be made by the introduction of a pump and treat system using activated carbon.
The biosensors were key tools in placing the conditions at the site in a context that can be used for predicting both environmental fate and remediation potential.

A13.43
The avian urate sphere, an ideal non-destructive biomarker of toxin exposure

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Urine is used clinically to monitor body metabolites, disease and other abnormalities. Whilst the acquisition of urine from wild animals is impractical, birds excrete urine as a white colloidal suspension of solid spheres, making field collection possible. The chemistry of these spheres (D.M. 65% urate, 5% albumin, 30% K and Ca cations) is critical to their structure but may contain trace contaminants without disruption; such as radioisotopes, heavy metals, toxins and phase II conjugates which are secreted in the kidney tubule along with urate. Sphere extraction makes use of their size (0.5–15 μm) and insolubility in alcohol by selective spirit filtration. To quantify sphere contaminants the urate concentration is determined, as in urine samples where constituents are measured against creatinine. For sample collection, larger birds provide adequate amounts and ground roosting birds minimise dispersal compared to cliff or tree roosts. Raptors have urate rich guano from their carnivorous diet; herbivorous birds ingesting bulky plant material add the white urate to the fibrous faecal pellet when passed; rain-wash removes the white urate from the guano.
Sampling vacated night roosts in daytime causes minimal interference. Wild birds are spatially (habitat) and temporally (season) distributed in the UK; behavioural (including roosting) habits also need to be considered when selecting a suitable species to use spheres from, as non-destructive biomarkers of environmental pollution. Applying this method we report ground roosting heather eating Red Grouse (*Lagapus lagapus scoticus*) had Cs-137 activity of 360 Bq/kg DM in extracted urate spheres (20% urate), compared with 480 Bq/kg (30% urate) in Black Game (*Lyrurus tetrix*) and 320 Bq/kg (53% urate) in Kestrels (*Falco tinnunculus*), on a North Pennine moor; the product of Chernobyl and nuclear weapons fallout.

A13.44
Comparison of sensitivity to Cadmium pollution in *Mnemiopsis leidyi* (Ctenophora), *Acartia clausi* (Crustacea) & *Gammarus* spp. (Crustacea)

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Mnemiopsis leidyi which belongs to the Phylum Ctenophora has caused many ecological problems after invading the Caspian Sea. It seems that the high decrease in Kilka, Sturgeons and Phoca population is related to the distribution of this Ctenophore in most regions of the Caspian Sea. It seems necessary to know about all biological and ecological features of *M. leidyi*. In this study the acute toxicity of the Cadmium Sulphate was determined to *Mnemiopsis leidyi*, *Acartia clausi* and *Gammarus* spp. and their LC_{50} for 96 hours were compared. The experiment was carried out in similar bottles with one litre filtered seawater and 10 individuals in each bottle. Our results show that the invasive Combjelly, *Mnemiopsis leidyi*, seemed to be more resistant to the $CdSO_4$ than *Gammarus* spp. and *Acartia clausi*. This difference was probably caused mainly by the dissimilar composition of these 3 species structures. This study indicates that high resistance of *Mnemiopsis leidyi* to the marine pollutions in comparison with other marine zooplankton may be the main reason for its high distribution in the Caspian Sea. It is strongly recommended that further studies of the tolerance of *M. leidyi* population to various environmental impacts, including oil products,

other heavy metals etc. be carried out and also can be compared with other marine zooplankton.

A13.45
Developmental effects of organic chemicals in harpacticoid copepods

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The ecological relevance and widespread distribution of harpacticoid copepods has led to them being widely used for assessing the effects of organic chemicals in both marine (*Tisbe battagliai*, *Nitocra spinipes*, *Amphiascus tenuiremis*) and freshwater (*Bryocamptus zschokkei*) environments. Most recently, harpacticoid life-cycle tests have been developed with the aim of evaluating the developmental and reproductive effects of chemicals where the main mode of action is to alter endocrine function in invertebrates or vertebrates. Harpacticoids are sensitive to a range of organic chemicals including natural oestrogens (17β-oestradiol), ecdysteroids (20-hydroxyedysone), pharmaceuticals (diethylstilbestrol) and pesticides (lindane and atrazine). The use of an 'in-vitro' ecdysteroid receptor based assay in conjunction with harpacticoid life-cycle data can also give further information on the mechanism of activity of the test compound. The small size and rapid generation time of harpacticoid copepods means that full life-cycle bioassays can be conducted in microplate test systems in as little as 21 days. These bioassays are both environmentally realistic and cost effective which has led to test guidelines using harpacticoids being adopted by ISO and OECD. Copepod bioassays are therefore recommended as screens for the chronic effects of potential endocrine disrupters in aquatic invertebrates.

A13.46
Successful detection of (anti-)androgens and aromatase inhibitors in pre-adult fathead minnows (*pimephales promelas*) using easily measured features of sexual development

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Screening assays have been successfully developed for the detection of (anti) oestrogenic substances in the fathead minnow (*Pimephales promelas*). However, a validated screen for detecting environmental (anti-)androgens and other endocrine active substances (EASs) has not been forthcoming. Our preliminary work suggested that pre-spawning adult fathead minnows might be an appropriate life-stage for developing a screen to detect these EASs. Pre-spawning adult fathead minnows, in which their phenotypic sex could be determined, were exposed (sexes separately) in flow-through systems, for 21 days, at 25°C. The three reference substances were dihydrotestosterone (DHT, androgen), flutamide (anti-androgen) and fadrozole (aromatase inhibitor). After 14 and 21 days exposure, fish were evaluated for growth, secondary sexual characteristics (number of nuptial tubercles), gonadosomatic index (GSI) and plasma vitellogenin (VTG) concentrations. Exposure to DHT increased the number of nuptial tubercles (male characteristic) in males (more abundant) and induced their appearance in females. Flutamide reduced nuptial tubercle number in male fish. Fadrozole inhibited ovarian growth (lower GSI) and induced testis growth. Plasma VTG concentrations were elevated in male fish, but inhibited in female fish, exposed to DHT. Flutamide had no effect on plasma VTG in male fish, but induced VTG in female fish. Fadrozole inhibited VTG in females and induced VTG synthesis in males. This work complements other published studies in supporting the current OECD effort towards validating a 21 day non-spawning fish screening assay for assessing (anti-)oestrogens, aromatase inhibitors and (anti-)androgens.

A13.47
The regulatory use of bioassays for effluent control – direct toxicity assessment (DTA) prioritisation of industrial discharges

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The Environment Agency has lead the way in applying Direct Toxicity Assessment (DTA) techniques in the UK for the last ten years. A corporate strategy has been implemented which includes a specific target to reduce the toxicity of point source industrial discharges known to have acutely toxic effects. DTA is considered a key approach to be used in achieving this target. Initially a baseline list of industrial effluent discharges with known, or with the potential for causing, acute toxicity in the receiving environment was derived using accepted DTA techniques. In compiling this shortlist, only the intrinsic hazard (measured toxicity) of each effluent was accounted for. In order to prioritise resources towards those industrial discharges with the potential to pose the greatest environmental risk, a simple numerical ranking system was developed and applied to the previously derived shortlist. The ranking system incorporated both DTA end-points and simple risk factors such as effluent

flow and dilution in the immediate receiving environment. Of the 24 short-listed discharges, the risk scores of six discharges were markedly greater then the remainder, indicating that they should be the focus of any further work. In the absence of a national effluent toxicity screening programme, this project has demonstrated that the techniques and methods prescribed in the Environment Agency's DTA Technical Guidance can be successfully used to target resources towards those discharges which, if improved, could result in the greatest environmental benefit.

A13.48
The effects of cadmium on the life history of the nematode *Caenorhabditis elegans*

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The effect of the heavy metal cadmium on the nematode *Caenorhabditis elegans* was investigated. Life-cycle toxicity experiments were conducted to examine the life history of worms exposed to increasing sublethal concentrations of cadmium. Life history traits under examination were survival, daily and total reproduction and growth. The short life-cycle of *C. elegans* facilitates full life-cycle analysis. The number of eggs laid decreased as cadmium concentrations increased and the length of the reproductive period increased in the lowest doses compared to control worms. Growth was measured throughout the egg, juvenile and reproductive period using digital image analysis and a lower growth rate was seen in exposed individuals. In conclusion cadmium has a significant impact on life-history traits in *C. elegans*. The most sensitive trait was reproduction, followed by growth and finally lifespan being the least sensitive of the measured traits.

A13.49
Mixed Species Toxicity Tests to Monitor the Impact of Leachate on an Aquatic Environment

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The treatability of a landfill leachate with respect to the removal of environmentally toxic components was assessed by acute and sub-lethal toxicity testing procedures. A specific leachate that had a $255 mgl^{-1}$ COD and $133 mgl^{-1}$ BOD was used for the research. Acute bioassays (LC_{50}) were undertaken using un-acclimatised juvenile *G. pulex* (pollution sensitive) and *A. aquaticus* (pollution tollerant) macro-invertebrates to establish pollution boundaries. The LC_{50} for *A. aquaticus* was 60% v/v leachate in clean water, whilst for *G. pulex* it was only 5%. Remediation techniques (air stripping, aerobic digestion and small-scale reed bed trials) were able to remove the toxicity of leachate towards *A. aquaticus* but *G. pulex* obtained a maximum LC_{50} of 90% v/v treated leachate in clean water.

Sub-lethal toxicity tests were carried out at concentrations that were lower than the acute toxicity thresholds for each species. The toxicity was judged on the basis of offspring produced and the growth rate of newly born individuals. As with the acute testing, it was found that *A. aquaticus* was relatively tolerant to the leachate compared to *G. pulex.* tests showed that a leachate dilution as high as 1:66 would affect the fecundity of the population of *Gammarus*, and even a dilution of 1:20 would influence the breeding colony size of *Asellus.* In contrast, sub-lethal investigations, which used treated leachate as a test media did not influence the fecundity or population size of either test species.

A13.50
Identifying potential metabolic biomarkers for withering syndrome in red Abalone (*Haliotis rufescens*) using metabolomic methods

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Withering syndrome (WS) is a fatal bacterial disease in abalone (ormers). It is associated with degenerative changes in the digestive gland that ultimately cause starvation, pedal atrophy, and death. WS has decimated black abalone (*H. cracherodii*) populations in California, and although red abalone have shown resilience when in relatively optimal conditions, studies suggest the disease can be synergistically stimulated by the presence of the pathogen in combination with elevated seawater temperature.

Here we describe a ^1H nuclear magnetic resonance (NMR)-based metabolomic investigation of WS in abalone, with an initial goal to identify biochemical markers for the disease. Hemolymph, foot muscle and digestive gland samples were obtained from three groups of abalone that were characterized as healthy, 'stunted' and diseased (based on morphological measurements). ^1H NMR spectra of these samples were recorded that contained hundreds of peaks corresponding to low molecular weight metabolites. Following spectral pre-processing, principal components analyses of the metabolite profiles were conducted. Our results confirm that metabolomics can successfully distinguish the biochemical

profiles of the three groups of abalone in all three types of tissue and biofluid. Furthermore, this discovery-based approach identified potential metabolic biomarker profiles associated with WS.

Most recently we have investigated the potentially synergistic effects of bacterial infection, temperature, and food availability on the metabolic status of red abalone. Preliminary analyses of the muscle and digestive gland NMR spectra again suggest that these environmental stressors (and combination of stressors) can be differentiated based upon molecular phenotype.

A13.51
High Throughput metabolomic studies of developmental toxicity in Japanese Medaka (*Oryzias latipes*)

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Developing embryos can be particularly sensitive to toxic insult. To identify the biochemical mechanism of action and the developmental stage at which toxicity occurs we require methods that can rapidly and inexpensively monitor the phenotypic changes during embryogenesis. ^{a}H nuclear magnetic resonance (NMR) spectroscopy-based metabolomics affords several advantages for such a study including minimal sample preparation, high-throughput analyses, and unbiased detection of low molecular weight metabolites.

We have investigated the metabolic changes occurring during normal development of medaka embryos. Several replicates at each of nine developmental stages were analyzed, covering the entire 8-day period of embryogenesis from fertilization to hatching. The ^{a}H NMR spectra were processed and analyzed using principal components analysis enabling visualization of the biochemical changes that occur between each developmental stage. This illustrates our concept of a 'developmental trajectory' through metabolic space, which summarizes the changes in the NMR-visible metabolome throughout embryogenesis.

Recently we have exposed embryos to a series of trichloroethylene (TCE) concentrations, a ubiquitous groundwater contaminant that is of concern as a developmental toxicant. Following continuous TCE exposure throughout development, embryos were analyzed on day-7 of embryogenesis, and a dose dependent metabolic perturbation was observed along with metabolic biomarker profiles associated with TCE toxicity. We are currently analyzing the results from a further study in which the phenotype of control and TCE-exposed (6 ppm and 800 ppb) embryos were followed throughout the entire period of embryogenesis. These analyses, along with the normal developmental trajectories of medaka will be presented.