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Evaluation of potential use of biomarkers as long-term monitoring tools in assessing ecological quality in terrestrial and aquatic environments of the UK

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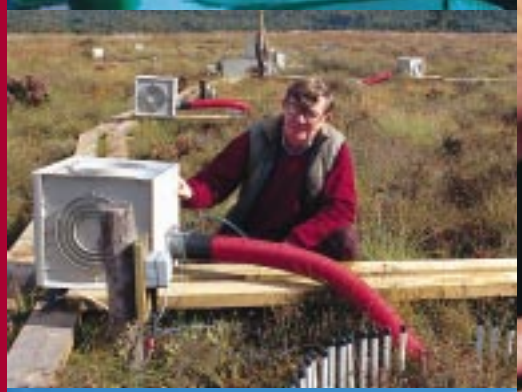

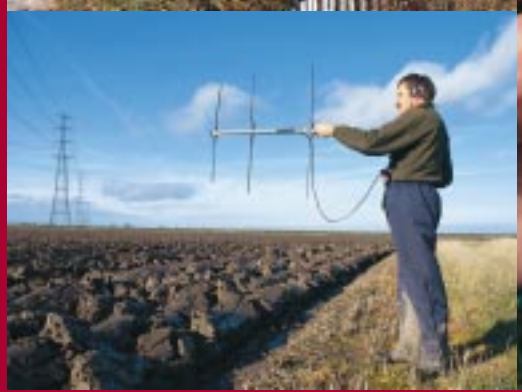

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English Nature Research Reports

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Evaluation of potential use of biomarkers as long-term monitoring tools in assessing ecological quality in terrestrial and aquatic environments of the UK

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Contents

1. Background

There is considerable advantage in using assessment and monitoring tools that are able to detect effects of chemical exposure in organisms and habitats before they become significant in conservation or ecological terms. Over the past couple of decades, there has been an increased emphasis on the use of biochemical, physiological and histological changes in organisms to provide information about exposure to chemicals and/or effects of exposure (Huggett and others 1992) Measurements of these changes are now generally referred to as "biomarkers". Biomarkers have potential application in assessing impacts on, or monitoring the condition of, living organisms, because they can provide evidence of exposure to chemicals (both individual compounds and mixtures of compounds), exposure to other stresses and/or an early warning of ecological impacts.

There are numerous definitions of biomarkers, including NRC (1987) and Peakall & McBee (2001). Principally, they all relate to measuring a change in biological effects, from molecular to behavioural, at or below the individual level, which demonstrates a change from the normal functioning of an organism.

English Nature used the following definition of biomarkers:

Biochemical, cellular, physiological or behavioural variations in the tissue or body fluids or at the level of whole organism that provide evidence of exposure to chemical pollutants, and may also indicate a toxic effect.

This is the definition we have used in this review and have included biomarkers such as scope for growth and histopathological changes.

There are advantages of using biomarkers in biomonitoring programmes. Biomarkers give a measure of bioavailability of a contaminant; demonstrating a biological change has occurred shows that the contaminant has been taken up and is having an effect on the biological functioning of an organism (Osborn and others 2000). Biomarkers give a measure of the effects of mixtures of chemicals (Fox 1993). A further advantage is that they may provide an understanding of the specific mechanisms of toxicity of a chemical. For example, induction of certain detoxification enzymes (eg cytochrome P450 1A) occurs through activation of the Ah receptor, which in itself may result in further changes. Biomarkers provide evidence of unexpected harm; by measuring changes in the biological responses we can determine the health of organisms within habitats of concern. A major advantage of using biomarkers for biomonitoring purposes is that they can show the effectiveness of remedial action. Biomonitoring programmes in North America measuring biological responses (in this case hepatic detoxification enzymes) have demonstrated that a reduction in organochlorine contamination resulted in an increase in the health of birds living in close proximity to a pulp and kraft mill (Sanderson and others 1994).

However, there are also limitations to using biomarkers, which need to be taken into account when using the data for biomonitoring purposes. These include the non-specificity of some biomarker responses. Some responses are not chemical-specific, for example lysosomal stability is affected by metals and organic compounds. Other responses are also induced by abiotic factors, for example heat shock proteins can also be induced through elevated temperature and anoxia as well as by xenobiotic chemicals. Some monitoring programmes

would benefit from the use of non-specific biomarkers, for example a general survey of the health of an ecosystem. However, other programmes may require the use of biomarkers that measure the effects of specific contaminants, for example monitoring the success of remedial action at a site of interest. The majority of biomarker responses are affected by biotic factors, for example age, sex and developmental stage, which need to be taken into account when interpreting the data. Some biomarker responses may be transient in nature so unless the timescale of exposure is known it may be difficult to relate cause and effect, such as cholinesterase inhibition following organophosphate exposure. The majority of biomarkers involve sampling organs, such as liver, kidney, and brain, and so are destructive in nature. This would mean that certain biomarkers are not suitable for monitoring rare and protected species. In such cases the use of biomarkers that are measured in body fluids such as urine or blood would be suitable. Many of the up and coming biomarkers have the potential to be non-destructive, for example, the CALUX reporter gene assay has been adapted and validated for use in blood samples (Murk and others 1997), which provides evidence of organochlorine and polycyclic aromatic hydrocarbon (PAH) exposure and gives a measure of the toxic equivalency factor of the chemicals present in the sample.

2. Scope of actual and potential use of biomarkers

An integrated approach to biomonitoring should be undertaken which includes both chemical and biological analyses. The Biomonitoring of Environmental Status and Trends Program (BEST) within the United States Geological Survey (USGS) recommended a suite of methods for monitoring effects of contaminants. These included chemical residues, biomarkers, toxicity tests and community-level impacts (T. Bartish, pers. comm. 2003). Furthermore, a suite of biomarkers covering the major functions of an organism should be measured (Fox 1993). The BEST program monitored a suite of biomarkers, including Cytochrome P450 1A enzymes, reproductive hormones, vitellogenin, immune function activity and histological examination of kidney, liver, gonads and spleen, were measured in fish (Schmitt and others 1999; Schmitt & Dethloff 2000; Schmitt 2002). The National Marine Monitoring Programme survey of UK coastal waters also monitored a suite of biomarkers which provided an indication of the general health of the coastal marine environment (Marine Pollution Monitoring Management Group 1998). In this way effects at different levels of physiological functioning of an organism can be determined. This also provides a general indication of the types of chemicals that are present in a habitat rather than focussing on one particular biomarker for a specific contaminant type in which the results may be inconclusive. Chemical residue analysis should also be conducted to obtain information on chemicals are present in the environment and at which concentrations that cause the effects. The 2000 Quality Status Report (OSPAR Commission 2000) also recommends monitoring of both chemical concentrations and biological effects to strengthen the information regarding long term ecological change. Continuous monitoring of habitats will ensure that any changes in the health of the area will be observed at an early stage before the effects begin to show at the population or community level. Many of these concerns with respect to monitoring have been mentioned in previous reviews, for example the Air Pollution Investigation System (APIS), OSPAR Commission and ICES reports.

It is also important to know the significance of a particular biomarker, i. e. can it be linked to higher-level effects? For example, imposex in dog whelk populations in coastal areas of Europe has been linked to a decline in the dog whelk population of these areas, which will affect the community structure. As toxicity of a chemical is initiated at the molecular level such responses are expected to provide an immediate measure of effect, occurring before

responses are observed at higher levels (Fox 1993). There are difficulties, however, in extrapolating from molecular effects to the whole organism, population and habitat. These links need to be further established to give us a greater understanding of the ecological relevance of the biological effects.

3. Objectives of the review

The main objective of this review is to provide English Nature with a comprehensive list of biomarkers currently in use, or that have the potential for use in biomonitoring of aquatic and terrestrial habitats within the UK, as outlined below:

Briefly "outline the state of readiness" of environmental sub-lethal biomarkers which could be used as long-term indicators of chemically induced stress on terrestrial and aquatic wildlife resulting from anthropogenic activities, and, in particular answer the following questions:

- What tools have been / are being developed?
- What is the range of circumstances these tools could be applied to?
- Which of the above tools are currently in use?
- What applications have been found for these tools?
- What potential applications are there for the tools identified above and how will they add to information derived from existing monitoring procedures?
- How could biomarkers be integrated into current or new monitoring programmes?

The main body of the review will be presented as two tables (These can be found on the enclosed CD). The first is a summary table that will provide a quick overview of the biomarkers, their advantages and limitations. The second table is more detailed and will enable searching upon items such as environment type. It is hoped that information provided in the second table will assist in the decision-making process regarding the potential for incorporation into new or existing biomonitoring schemes. In addition, a workshop, represented by key organisations, was held in September 2003 to discuss this review and assess the practical application of using biomarkers as monitoring tools. The proceedings of this workshop are summarised in **Appendix 1.**

4. Criteria used to assess biomarkers

There are certain criteria that must be considered when using biomarkers for monitoring contaminated habitats. Improper application or interpretation has severe consequences as both false negatives and false positives could be inferred (Svendsen 2001).

The criteria set out below have been incorporated into the table, together with other information including environment type, tissue type, advantages and limitations. Examples of current use either for research or routine biomonitoring purposes, within the UK, Europe and North America, for each biomarker, have been added. Also included in the table are some reporter gene, novel bioassays (located at the bottom of the tables) that were thought to be promising tools for biomonitoring purposes. These particular bioassays were included as they provide evidence of the mechanism of toxicity of particular classes of chemicals (ICES 2001), for example a positive response from the ER-CALUX assays means that compounds

are present that are active via the oestrogen receptor (ICES 1999). Other bioassays, such as whole sediment bioassays, microbial assays and water bioassays, were not included as they are not chemical (class) specific and tend to provide a measure of general toxicity.

4.1 Response characteristics

4.1.1 Exposure dose-response relationship

The degree to which the magnitude of the response depends upon the dose of the exposure must be known. It is of course desirable that there is a clear relationship between the degree of exposure and that of the resulting response, so that the severity of the exposure can be assessed directly.

4.1.2 Exposure time-response relationship (inducibility & persistence)

The time it takes from the onset of exposure until a response is manifested, and the time the response persists after exposure ends, are both parameters that have a large impact on the interpretation we can and cannot place on a given biomarker response. The response time-course of biomarkers varies widely from nearly instantaneous (eg stress proteins) to years (eg cancerous lesions). Depending on the objective(s) of a study, rapid or slow response times may be desirable (eg early warning or demonstration of prolonged exposure). The question of the persistence of biomarker responses involves the issues of transience and reversibility of the response. Some biomarkers have transient response patterns and will be induced for a certain period of exposure before returning to lower or even normal levels although exposure is continued (eg induction of glutathione S-transferase). Reversibility of the responses of non-transient biomarkers varies from complete (ie reversible) to none at all (ie permanent).

4.1.3 Inherent variability (other confounding factors)

This review included information on potentially confounding factors such as reproductive stage, sex and age, as well as temperature and season. These may affect the response but in themselves do not actually induce the response (see the column 'additional stressors that activate the response'). An example of that is reproductive stage and season are thought to affect the cytochrome P450 1A response in organisms. These therefore need to be taken into account when interpreting the data. To control for these factors it would be ideal to have a 'clean' reference site to compare responses, thus enabling the effects of exposure to be isolated. Alternatively, if non-chemical factors exist that cause large changes in the response-patterns they must be well understood so that misinterpretation can be avoided, ie comprehensive base-line data is required.

4.2 Sensitivity

The sensitivity of biomarkers should be higher than that of life-history parameters for the biomarker to be most useful. High sensitivity could originate from either a very short response time from first exposure or the ability to detect changes at lower concentrations. In this review sensitivity is related to responding to low concentrations of the contaminant, short response time is covered in the general response characteristics section.

4.3 Biological specificity

Some biomarkers are more applicable to certain groups of organisms for various reasons. General physiological differences might dictate whether the required amount of sample can be obtained, or even if the sample material is available in certain organisms.

4.4 Chemical specificity

Biomarkers are in general divided into non-specific and specific biomarkers on the basis of what they respond to. Non-specific biomarkers give a measure of general stress resulting from a broad range of pollutants (eg several classes of pollutants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), etc.), whereas specific biomarkers measure effects that can be linked directly to a specific pollutant (eg DNA adducts).

4.5 Ecological relevance (Links to effects at higher levels)

The utility of biomarkers as predictive tools is based on there being some link between the biomarker response and effects at higher levels of organisation, or the likelihood of such effects occurring. Lack of such linkage, either correlative or causal, may just be a consequence of the current state of understanding for a particular biomarker. Whilst the lack of linkage does not invalidate the use of the biomarker, it will limit its predictive potential and therefore its use in environmental decision making.

4.6 Explanation of costs

Low – Method can be conducted using equipment available in a standard research laboratory such as microscopes, spectrophotometer, fluorimeter and basic electrophoresis equipment. There is a high sample throughput resulting in low staff costs and the analysis and data interpretation is straightforward.

Medium – Method can be conducted as above. There is low sample throughput resulting in high staff costs. More complex data analysis and interpretation.

High – Method requires the purchase of special equipment and consumables are expensive. There is low sample throughput and analysis and data interpretation requires specialist skills and expertise.

5. Conclusions

It was not requested to recommend specific biomarkers in this review, this conclusion will re-iterate salient points mentioned previously. An integrated approach should be adopted for the use of biomarkers in biomonitoring schemes, which includes measurement of biological effects and chemical residue data to provide information about the extent of contamination to habitats of interest. Biomarkers should be used as a complementary tool rather than as a stand alone measure to determine the extent of contamination within an environment. A suite of specific and non-specific biomarkers covering a range of biological responses from molecular to whole organism changes should be used as screening-level indicators of widespread contamination. Some programmes would benefit from the use of non-specific biomarkers while other, more focussed programmes, including those monitoring the success of remedial action at certain sites, would use biomarkers that are targeted at specific pollutants.

Continued monitoring of ecosystems should be undertaken to measure the success of remedial action and for early warning of potential adverse effects before population and community level changes occur.

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Appendix 1

Proceedings from the Workshop on the Potential Application of Biomarkers for Environmental Monitoring in the UK

22-23 September 2003

1. Presentations

1.1 Background to Meeting and English Nature's interest in biomarkers – Michael Coyle, English Nature

Michael Coyle gave a presentation describing the rationale behind English Nature commissioning the biomarkers review and the subsequent workshop.

English Nature covers a wide remit as a statutory body that champions the conservation and enhancement of the wildlife and natural features of England. Through the Joint Nature Conservation Committee, English Nature also has a lead agency role with respect to the provision of advice on pesticides and toxic substances. There is therefore a requirement to improve understanding of the potential impacts on wildlife from exposure to anthropogenic pollution.

The overall objective of the commissioned study was to carry out a broad review of biomarkers, and their "state of readiness" for the purposes of assessing risks to habitats and wildlife in the marine, freshwater, and terrestrial environment. The information will enable English Nature to assess the potential of these tools in current and future monitoring programmes.

The aim of the workshop was to:

- Present and receive feedback on report.
- Initiate discussion on the potential application of biomarker tools.
- Discuss the way forward.

1.2 Biomarkers report – Sara Long, CEH Monks Wood

Sara Long (Contract Manager) gave a presentation describing the objectives and methodology of the study.

The specific objectives of the review were to address the following questions:

- What tools have been/are being developed, and what is their "range"?
- Which of these tools are currently being used and what is their application?
- What is the potential for all tools identified?

The main sources for the report were:

- Recent reviews for both terrestrial and marine environments.
- Websites produced by national organisations concerned with environmental assessment.
- Workshops & Conference Proceedings.
- Literature searches.

The report mainly consisted of two tables; a summary table that describes the biomarkers listed in non-standardised terms to provide descriptive information and the second a more detailed table with standardised inputs facilitating easy interrogation.

1.3 Specific application of biomarkers – Michael Coyle

Three main areas of English Nature's work were identified where information from biomarkers could potentially be used:

- Condition assessment of sites designated for nature conservation under national and European legislation[1.]
- Assessing impacts from plans and projects on sites designated under the Birds and Habitats Directives, as a requirements of the Habitats Regulations 48 and 50 (1994) [2.]
- Assessment of wildlife "health" in the wider environment eg JNCC wildlife and pollution contract.

Both judgments of favourable conservation status[1] and "no adverse effect on integrity"[2] need to address long term sustainability of the structure and functioning of the site.

Workshop participants were asked to discuss:

- What role could biomarkers have in these impact and condition assessments?
- Do they need to relate to population-level effects to be useful?
- Would biomarker information help or "muddy" assessments?
- How could they be used as a rapid, cost-effective screen for assessing impacts and risks on sites and the wider environment?

Notes:

1. The Habitats and Birds Directives require member states to designate habitats and species of conservation importance and aims to maintain or restore habitats and species to favourable conservation status.

2. Plans and projects must be assessed to ensure that they are not having an adverse effect on the integrity on the site, alone or in-combination with other plans and projects.

1.4 Predatory Bird Monitoring Scheme (PBMS) - Richard Shore, CEH Monks Wood

The PBMS is a long running national monitoring scheme run by CEH Monks Wood, and part funded by the Joint Nature Conservation Committee (JNCC). The aim of the scheme is to quantify exposure to pesticides and industrial contaminants in predatory birds. The methodology, and critical aspects of the scheme were outlined.

Workshop participants were asked to consider the following questions:

- Are there are current biomarkers that could be used in such a scheme?
- Are there novel/potential biomarkers that should be used?
- What are the key up and coming approaches?
- What would biomarker information add to the value of the scheme?

2. General discussion and key messages arising from workshop

2.1 Biomarkers - principles of use

2.1.1 Before biomarkers are deployed in the field, it is essential that there is a clear understanding of the scope and nature of the intended monitoring, and the context in which any results will be used.

2.1.2 Three main roles for biomarkers were identified: A **screening** tool that could trigger further study of a site, a **diagnostic** tool which focuses on specific issues identified, and a **monitoring** tool to follow the 'health' of a site over time, perhaps following changes in policy or site management to monitor remediation success.

2.1.3 In principle, biomarkers with clear linkage to higher order effects, such as at the population, community and ecosystem function level, would be the most effective at demonstrating to stakeholders and regulatory bodies that further action is required.

2.1.4 The effective use of many biomarkers is dependent upon good baseline knowledge of the natural variation in that biomarker response. Although reference information may not be necessary to follow a time trend in biomarker response, the ability to interpret those changes is limited without such background information.

2.1.5 In most cases, information from individual biomarkers is unlikely to be robust enough for regulatory action to enforce a change in management of a site, although a suite of biomarkers could provide a body of evidence. For example a suite of biomarkers for an estuarine system could include scope for growth (a measure of growth), vitellogenin induction in male fish, frequency of occurrence of inter-sex (both measures of reproductive health), and DNA-adduct formation (a measure of the health state of a population).

2.1.6 The effective use of a suite of biomarkers is dependent upon a cohesive framework for the use of different biomarkers and clear actions to be taken in response to the suite of results produced.

2.1.7 There is a requirement for sub-individual measures that would, when a negative response is measured, provide reassurance of no adverse impacts and, when the response is positive, trigger further investigations.

2.1.8 Some of the key considerations before deploying biomarkers include a knowledge of the of sensitivity of responses, access to standardised methodology, reproducibility of the techniques, and the ability to assess how comparable results are. This aspect of quality assurance of methodologies is an increasingly fundamental requirement for monitoring schemes.

2.2 Present challenges of applying biomarkers as monitoring tools

2.2.1 In general, links between biomarkers and population level effects are unclear, although there are some examples where the liks are established such as David Spurgeon's research on the Avonmouth smelter (Spurgeon and others 2004) and tributyl tin (TBT) and imposex in molluscs (Gibbs and others 1987).

2.2.2 A major challenge was centred on convincing policy makers in agencies that a biomarker response necessitated management or regulatory action. Perhaps biomarker responses could be converted to a traffic light system to get the message across to experts in other disciplines and to stakeholders.

2.2.3 The workshop participants discussed whether precautionary action could be based on exposure biomarkers alone or whether effect biomarkers were required. It was acknowledged that although exposure biomarkers lend themselves to certain surveillance monitoring, effects biomarkers would add considerable weight to providing evidence of harm and are more influential in considering management action.

2.2.4 The definition of what would constitute "harm" requires further thought. The main initial English Nature requirement was for tools that helped site assessment where health of a site would equate to the functional and structural integrity of populations, communities and ecosystems within a single or meta-population of sites.

2.2.5 Some monitoring schemes operate a passive monitoring approach, for example the PBMS in which carcasses are submitted to the scheme by members of the public for chemical analysis. This approach would severely limit the choice of biomarkers as most require fresh material. Therefore at present most biomarker-based monitoring would have to be active, ie targeted sampling of fresh material. An active monitoring scheme that relies upon destructive sampling may lead to conservation concerns for rare species and so non-destructive sampling would be desirable unless a common surrogate species could be used.

2.2.6 At present the application of biomarkers to monitor terrestrial systems lags behind those applied to the marine environment. However, rapid progress may be made through the EA's contaminated land projects.

2.2.7 Caution should be used when interpreting spatial or temporal trends in biomarker responses. A good understanding of the dynamics of response is necessary, for

example in the latency of a biomarker response, ie, the duration of a biomarker response in relation to exposure

2.3 Factors affecting tool selection

2.3.1 The most useful biomarkers are those which indicate the state of a site or key organism, in relation to key indicators such as "death", "growth", "reproduction", "disease state" and/or "alive and well" (healthy).

2.3.2 There is a need for biomarkers from which the chain from lower order effects (eg biochemical or molecular) to population level effects is short.

2.3.3 The use of surrogate species may be especially desirable if there are conservation concerns associated with the target species. This could facilitate a more standardised methodology and, if the surrogate species was common and widespread, would allow for inter-site comparisons.

2.3.4 In order to choose a suite of biomarkers which cover the appropriate range of response types and stressors, conceptual models need to be developed to target potential risks on specific sites.

2.3.5 English Nature has developed favourable condition tables which are used to supplement conservation objectives and inform the condition assessment of SACs and SPAs. These objectives comprise of high-level indicators of condition, although other contextual information such as water quality will be considered in any assessment. Biomarker responses could be used to validate the ecological relevance of water quality data eg EQS failure, and also to address some of the limitations of only using EQSs for water quality management. It is envisaged that a suite of robust and relevant biomarkers could potentially contribute to condition assessments.

2.4 The way forward

2.4.1 There is a need to develop methods for biomarker measurements into standardised tests that have been ratified by international organisations such as the Organisation for Economic Co-Operation and Development (OECD) and the International Organisation for Standardisation (ISO) tests.

2.4.2 There is a need for research to concentrate on bringing a smaller number of more robust biomarkers to the stage where they could fit into a framework of assessment. Within this suite of biomarkers there is a need for both general health biomarkers and also biomarkers that are mechanistically linked to higher order effects.

2.4.3 Similarly, like current OECD and ISO tests, biomarkers could fit within a tiered framework for assessing sites. An example of the use of a tiered approach is the use of biomarkers from the contaminated land regime of the EA. A tiered approach may be as follows:

a. Tier 0 – Biological surveillance to stratify resources and answer whether some precautionary work is required

b. Tier 1 – The employment of non-specific biomarkers to identify deviation from normality.
c. Tier 2 – Specific biomarkers that are related to reproduction or growth for example to establish cause and effect.

2.4.4 The relevance of experiences obtained from the medical research community to ecological applications should be considered ie what are the effects in human health monitoring which act as compelling arguments/triggers (red lights) for further action? Reproductive effects, such as embryotoxicity, or oncological effects, are strong triggers for further action in human health. It was envisaged that biomarkers that predict these types of effects in environmental monitoring would be important.

2.4.5 Transcriptomic technologies utilise multivariate statistical approaches that are good at identifying outlying samples, even when the background noise (natural variation) is large. This approach could possibly be applied to responses from a suite of biomarkers to identify sites that require further study, ie aiding the identification of priority sites of concern and be the trigger for further action.

2.4.6 Once an effect/problem has been identified using a biomarker, bioassays may be an informative and cost effective way of assessing the significance in any response.

2.4.7 If effects measures could be presented as frequency of occurrence in a population, representing either presence or absence of response or number of responses above a critical level, this would constitute a clearer message to policy makers and stakeholders.

2.4.8 It would be beneficial for the science community to start to build up a database for a suite of biomarkers which respond to a wide range of stressors. Ideally a central / open database for results of biomarker trailing studies including details such as species, environment and site characteristics would be helpful.

2.4.9 Establishing mechanistic links from biomarker responses to reproductive and oncological effects (eg oocytes in testes or tumours in fish), and/or the utilization of frequency measures of on-off responses give a powerful message to policy and regulatory bodies, which in turn are easily understood by other stakeholders including the general public.

2.4.10 Other biomarkers which could be used in areas where there are conservation concerns are those that are measured in samples taken non-destructively, ie in body fluids and through biopsies. Presently non-destructive biomarkers are not in a state to be routinely used, although the work of Christina Fossi and colleagues is improving our understanding of these techniques (Fossi and others 1992, 2000 & 2003). The techniques include measuring cytochrome P450 and MFO enzyme induction from skin biopsies taken from marine mammals.

2.4.11 To develop biomarkers for use within an assessment framework it is necessary to trial these tools on a well characterised site, ideally with a gradient of exposure in order to test their sensitivity. An example of this is work carried out around the Avonmouth smelter establishing links between molecular and sub-cellular responses and population level effects along a metal gradient.

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Top left: Using a home-made moth trap.
Peter Wakely/English Nature 17,396
Middle left: Co_2 experiment at Roudsea Wood and Mosses NNR, Lancashire.
Peter Wakely/English Nature 21,792
Bottom left: Radio tracking a hare on Pawlett Hams, Somerset.
Paul Glendell/English Nature 23,020
Main: Identifying moths caught in a moth trap at Ham Wall NNR, Somerset.
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