

National Centre for Environmental Monitoring
&
Surveillance

Environment Agency

Biosensors for Environmental Monitoring:
A review of capabilities and strategies for the
future



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*National Centre
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1. INTRODUCTION

1.1 Background

Biosensors are a class of molecular sensors that have enjoyed much recent success in the clinical diagnostics field. Recent developments suggest that they are now poised to exert a similar impact on environmental monitoring practices. Their use of biochemical reactions, combined with modern sensor technologies and electronics, confers the potential to quantify rapidly and cost-effectively a wide range of analytes of environmental interest.

It has long been recognised that biosensors may offer prospects for use by the National Rivers Authority and the forthcoming Environment Agency. Despite some preliminary work by the NRA, no comprehensive appraisal has been made of their potential. This study makes such an appraisal and proposes a strategy for the development and deployment of biosensors.

1.2 Objectives

The prime objectives of this study are to familiarise the NRA with the present-day and anticipated future capabilities of biosensors and to propose a strategy whereby it could best and most usefully become involved with the technology. The study's specific objectives are to:

1. elucidate the underlying principles and technologies;
2. identify the analytes that may be measured with biosensors;
3. provide details of available and forthcoming products;
4. review relevant, current research;
5. provide details of the activities of key research groups;
6. forecast future capabilities of biosensors, including perceived benefits and limitations, and timescales for development;

7. locate any biosensor activities underway by other UK organisations and investigate scope for collaboration;
8. review the US EPA's biosensor R&D programme;
9. investigate biosensor activities by other European environmental agencies;
10. investigate the implications of standardisation;
11. analyse the potential role of biosensors within the NRA and the forthcoming Environment agency, including a consideration of the operational and economic benefits that could be derived;
12. propose a strategy whereby the NRA/Environment Agency could move forward, with an emphasis on collaborations, stimulating manufacturers to develop products that meet the NRA/Environment Agency requirements, and supporting academic or corporate R&D.

1.3 Scope

This study considers biosensors as defined in 2.1, below. Only those analytes, products, technologies and research activities relevant to the interests of the NRA and the forthcoming Environment Agency are considered.

1.4 Method and data sources

This study was undertaken principally by equating the perceived sensing and measurement requirements of the NRA and the forthcoming Environment Agency, with the actual and anticipated future capabilities of biosensors.

Much of the technical information was derived from:

- the recent research literature;
- conference proceedings;
- previous reports and surveys;
- product literature.

Published literature referred to in the text is listed under "References" at the end of this report.

Discussions were held with individuals from:

- university and corporate research institutions;
- the NRA and HMIP;
- other UK and overseas bodies with an interest in biosensors;
- biosensor manufacturers and distributors;
- standardisation bodies;
- overseas environmental agencies.

The affiliations of the individuals who contributed to this study are listed in Appendix A.

1.5 Abbreviations

The following abbreviations are used in this study.

- BOD - biochemical oxygen demand
- EA - Environment Agency
- ELISA - enzyme linked immuno-sorbent assay
- FIA - flow-injection analysis
- FOS - fibre optic sensor
- IA - immunoassay
- IOS - integrated optical sensor
- NRA - National Rivers Authority
- PCB - polychlorinated biphenyl
- ppm - parts per million
- ppb - parts per billion
- ppt - parts per trillion
- SPR - surface plasmon resonance

1.6 Acknowledgements

The contributions of the many individuals who assisted in the compilation of this study are hereby gratefully acknowledged by the author.

2. BACKGROUND, PRINCIPLES AND TECHNOLOGY

2.1 Definition

A biosensor is a sensor in which the analyte reacts with a biological agent (see 2.3, below) producing some change which is quantified by a transduction device that generates a functionally related output signal. The biological agent is in physical contact with the transducer, thus, a biosensors may be viewed as:

"A sensor deriving its output from an interaction between the analyte and a biological agent, in contact with a transducer".

The term, therefore, simply defines one aspect of the sensor's construction (the use of a biological agent), rather than any of the more widely used means of classifying sensors such as underlying technology (silicon, thick-film, fibre optic), measurand (flow, pressure, pH), or application (medical, automotive, environmental).

Measuring devices involving biological agents that are not in contact with a transducer are not biosensors according to this definition. Examples are instruments and procedures using non-immobilised biological agents such as respirometers, immunoassay-based test kits and enhanced chemiluminescence tests.

2.2 Historical perspective

Biosensors were first described by Leland Clark at a symposium of the New York Academy of Science in 1962, where the use of enzymes were proposed to improve the performance of electrochemical sensors (Clark and Lyons 1962). Thus was born the enzyme electrode and in the following three decades, all manner of biosensors have been investigated and developed, and some taken to market.

By far the greatest success has been achieved in the medical sector, which provides the most outstanding success story to date: the MediSense blood glucose "test strip". Whilst deceptively simple in appearance and operation, this planar thick-film biosensor is a triumph in immobilised biochemistry and mass-production technology. Based on ferrocene-mediated, amperometric determination of the glucose oxidase reaction, this sensor represents the culmination of many years of fundamental research by Cranfield Institute of Technology (now Cranfield University) and the University of Oxford. Today, several million test strips are produced each month in the UK.

Medical applications dominate the present-day biosensor market and only in more recent years has the potential role in environmental monitoring been investigated. The realisation that highly sensitive and selective techniques such as immunoassay and enzyme reactions could be applied to detecting "environmental" analytes has led to a mushrooming of R&D activity, with the result that a small number of biosensors are now available commercially for this use (see section 3).

The markets for biosensors are still comparatively small but are growing rapidly. According to a recent survey (Cranfield Biotechnology 1991) the World market will be worth \$360 million in 1996 and reach \$700 million by the year 2000. The environmental sector of this market represents only a small part of the whole: Cranfield estimates that this will be worth a mere \$15 million in 1996 but will rise to around \$100 by 2000. To place these figures in perspective, the market for silicon sensors in Western Europe alone was worth \$373 million in 1993 and is forecast to reach \$670 million by 1998 (Frost & Sullivan 1993). Global markets are valued at around three times these figures.

The recent research literature describes biosensors for over 120 analytes but commercial devices are available for only around 10-15. This fact alone confirms the widely held view that their potential has hardly been tapped.

2.3 Biological agents and effects

2.3.1 Biological agents

The so-called "biological agents" that characterise biosensors react with the analyte to produce the change that is quantified by the transducer. They are derived mostly from living systems and examples are listed in Table 2.1.

TABLE 2.1 - Biological agents used in biosensors

living micro-organisms (bacteria, yeasts, plants, slime moulds etc.)
cellular components (chloroplasts, cell membranes etc.)
plant and animal tissues
enzymes
antibodies
nucleic acids
other biological molecules (haemoglobin, hormones, lipids etc.)

2.3.2 Biosensor phenomena and effects

Many different biochemical reactions and phenomena are, or may be, exploited in biosensors. Whilst in some instances, these produce a change that can be detected directly by the transducer, for example, the oxygen consumption of a colony of micro-organisms measured with a dissolved oxygen electrode, it is more common for various intermediates to be used. These convert the results of the primary interaction into a quantity that can be determined by the transducer.

A diverse range of effects are employed in the transducers to convert the results of the biochemical reactions into an electrical or optical output signal. Examples of biochemical phenomena and transducer effects are listed in Table 2.2.

TABLE 2.2 - Biochemical phenomena and transduction effects

<u>Biochemical phenomena</u>	<u>Transduction effects</u>
immunochemical reactions	resistive
enzyme reactions	conductimetric
respiration rate	potentiometric
reaction inhibitions	amperometric
bioluminescence	calorimetric (measuring heat of reaction)
chemiluminescence	optical (attenuation, fluorescence, SPR etc.)
cellular activity	acoustic (eg. change in wave velocity)
toxic responses	gravimetric (mass loading effects)

2.4 Biosensor technologies

In view of the wide variety of biological agents, biochemical phenomena and transduction principles that can, potentially, be employed, it is no surprise that biosensors may be based on many different sensor technologies or combinations of technologies.

The literature contains references to prototype devices based on almost all existing sensor technologies and some examples are illustrated in Table 2.3. This technological diversity largely reflects that, as yet, most devices are still at the research stage and consequently, a very wide variety of technologies are being investigated.

TABLE 2.3 - Biosensor technologies

<u>Technology</u>	<u>Examples</u>
Thick-film	Thick films of enzymes deposited on ceramic substrates by screen and ink jet printing technologies, with thick- or thin-film sensing electrodes.
Thin-film	LB and other thin films of biological agents deposited on optical fibres, IO structures etc.
Silicon	Enzyme layer deposited on silicon substrate with thin-film sensing electrodes, silicon "BioFETs", "EnFETs" and "ImmunoFETs".
Fibre optic	Optical fibres coated with films of biological agents, interactions with the evanescent wave, FO detection of bioluminescence, etc.
Integrated optical	Luminescent reactions detected with IO devices, other surface reactions by SPR, refractive index changes, interferometry, etc.
Electromechanical	Biological agents immobilised on piezoelectric surface acoustic wave (SAW) and resonating devices
Glass electrodes	pH and ion-selective electrodes with membranes coated or impregnated with biological agents

Broadly speaking, silicon and thick-film technologies are the strongest candidates for low cost, disposable biosensors, particularly for field uses, and are proven in the medical sector. Optical technologies (IO, FO), offer good prospects for high accuracy, re-useable sensors by virtue of the use of highly sensitive phenomena such as SPR and interferometry. However,

these are generalisations only and as with all classes of sensors, the technology that is ultimately adopted is determined by an often complex and interactive combination of operational and economic factors.

3. PRODUCTS

3.1 Available products

As previously noted, in contrast to the medical sector, very few biosensors are yet available for environmental monitoring applications.

This largely reflects the fact that biosensor developments are both time consuming and costly: British Gas's MEG biosensor (see below) took six years to develop and Pharmacia's SPR-based "BIACore" took ten years, at a cost of around \$100 million. Thus, before any company makes such a commitment, significantly sized markets, or those commanding high prices for low volume, high value products, need to be identified. Furthermore, few companies involved with environmental monitoring instrumentation have sufficient resources to undertake such developments in-house.

A list of available environmental biosensors is given in Table 3.1 (overleaf) and further details are provided in Appendix B. Almost all are the result of earlier, academic research or collaborations between manufacturers and universities.

TABLE 3.1 - Commercially available environmental biosensor products

<u>Manufacturer</u>	<u>Product</u>	<u>Analyte</u>	<u>Principle</u>
Bruno Lange (Germany)	SensorBOD	BOD	Oxygen consumption of colony of micro-organisms
Central Kagaku (Japan)	BOD .2000 BOD 2200	BOD	Oxygen consumption of colony of micro-organisms
Medingen (Germany)	BSB Module	BOD	Oxygen consumption of colony of micro-organisms
Biosensori (Italy)	MIDAS PRO	total viable cells	Mediated amperometry
SERES (France)	Pesticide Analyzer	triazines (on-line)	Immunoassay with colourimetric detection
British Gas (UK)	MEG Biosensor	monoethylene glycol	Enzyme reaction/amperometry

3.2 Products under development

The above table illustrates that many of the available products are aimed at the rapid measurement of BOD. However, whilst summarising the present situation, this table provides a poor insight into the potential offered by biosensors in the short to medium term. Current commercial developments illustrate far better their likely future prospects and summary details of a number of these are illustrated in Table 3.2.

TABLE 3.2 - Some commercial biosensor developments

<u>Company</u>	<u>Analyte</u>	<u>Principle</u>
Ohmicron (US)	pesticides, organics, metals	Immunoassay/electrochemical detection (field sensors)
GEC-led consortium (UK)	pesticides in water	Immunoassay/optical techniques (BIOPTICAS project)
Hewlett-Packard (US)	pesticides in water	Immunoassay/surface transverse wave sensor
Cell Diagnostica (Germany)/Cranfield Diagnostics (UK)	pesticides in soil	Immunoassay/amperometric detection with organic sample extraction
Undisclosed German company (with GBF)	pesticides in water (on-line)	FIA system using competitive immunoassay
Lumac (Netherlands)	heavy metals	Genetically engineered live cell (injected lux genes)/optical
Aquateam (Norway)	coliform bacteria	Enzyme hydrolysis/fluorescence
Microbics (US), Siemens-Plessey (UK)	toxicity (on-line)	Toxicity-induced reduction in bioluminescence from bacteria
Ecossensors (UK)	toxicity and BOD (in field)	Oxygen consumption of colony of micro-organisms

(Continued overleaf)

Table 3.2, continued

<u>Company</u>	<u>Analyte</u>	<u>Principle</u>
Bookham Technology (UK)	various environmental analytes	10 evanescent wave technology
Omega SRL (Italy)	micro-organisms in water	pH change in silicon ISFET
City Technology (UK)	toxic gases (SO ₂ , CH ₄ etc.)	Enzyme reactions/thick film microelectrodes

4. BIOSENSOR RESEARCH

A reasonably detailed knowledge of prevailing environmental biosensor research is necessary to gain a meaningful insight into future capabilities. This section is, therefore, through necessity, somewhat lengthy and technical.

4.1 Overview

4.1.1 Scope of research

Biosensor research is underway in most Western European countries, Eastern Europe, Russia, Japan, the Far East, the US and elsewhere. The growing recognition of the potential role of biosensors in environmental monitoring is illustrated well by the fact that many university research groups that concentrated formerly on medical uses are now turning their attention towards environmental applications.

Research addresses a diversity of both fundamental and practical issues, as illustrated by Table 4.1.

TABLE 4.1 - Scope of environmental biosensor research

Biological agents
Biochemical reactions and sensing phenomena
Transduction effects
Immobilisation techniques
Fabrication technologies
Signal processing
Calibration
Selectivity and sensitivity
Reproducibility
Shelf life/stability

The most intensively studied biochemical phenomena in this context are immunological and enzyme reactions, thus, the most widely used biological agents are antibodies and enzymes. The transduction techniques under study are far more varied and include a diversity of optical and solid-state phenomena, as illustrated in Table 2.3, above.

4.1.2 Potential applications

It is important to recognise that research aims to develop biosensors that respond to several classes of analytes in a variety of environments, ie.

- chemical species in the liquid phase;
- microbiological species in the liquid phase;
- gases and vapours;
- biologically-active airborne solids (eg. enzymes etc.);
- chemical species in the solid phase (eg. soil).

Furthermore, applications are not restricted to water analysis with portable or laboratory instruments. Several groups are developing on-line analysers, which are likely to be of interest to that part of the forthcoming EA responsible for Part A process emissions and Red List species (now HMIP), whilst others are investigating soil analysis, perhaps of greatest interest to the waste regulation side. A small body of work is involved with gas detection, although applications in the short-term are probably more likely in occupational health and safety than environmental monitoring.

4.1.3 Analytes

The research literature refers to prototype biosensors that respond to numerous analytes of environmental interest, as listed in Table 4.2. The majority are species encountered in water, whose field determination is prohibitively slow, complex, costly or impossible. Some, such as MEG and TNT, are associated with highly specialised applications.

TABLE 4.2 - Environmental analytes

<u>Liquid phase</u>	<u>Gas phase</u>
BOD	sulphur dioxide
pesticides (herbicides, fungicides, insecticides)	methane
pesticide transformation products	airborne biochemicals
metals (Cu, Cd, As, Zn, Co, Hg, Cr etc.)	noxious odours
nitrate, nitrite	phenol vapour
phenols, nitrophenols	carbon monoxide
sulphite, sulphide	
chlorinated phenols (PCBs etc.)	<u>Solid phase (soil)</u>
ammonia/ammonium	PCBs
toxicity	pesticides
cellular biomass	metals
bacterial numbers	
bacterial species and groups	
trinitrotoluene	
phosphates (inorganic)	
silage liquor	

The following sections consider certain specific fields of biosensor research and are arranged according to analyte. Sections are devoted to analytes that are likely to be of the greatest interest to the NRA/Agency and which are the topic of a significant degree of potentially exploitable R&D. The following are considered:

- pesticides;
- BOD
- toxicity;
- phenols;
- gases;
- other species (metals, PCBs, micro-organisms, silage).

A short section is devoted also to soil analysis.

4.2 Pesticides

4.2.1 Research overview

Pesticide detection is the most active field of environmental biosensor research and several different principles are under study. The most widespread are antibody-based immunochemical reactions and the inhibition of enzyme reactions (eg. for organophosphorous and carbamate species), with a smaller body of work on various live cell approaches. Many different optical and solid-state transduction mechanisms have been used in conjunction with these.

Some of the many pesticide species detected with prototype biosensors include:

- | | |
|-------------------|------------------|
| - Simazine | - 2,4-D |
| - Atrazine | - 2,4,5-T |
| - Propazine | - Terbutryn |
| - Diuron | - Carbofuran |
| - Chlorfenvinphos | - Malaoxon |
| - Isoproturon | - Paraoxon |
| - Fenitrothion | - Fenamiphos |
| - Temephos | - Pyridafenthion |

The stimuli behind this work are twofold: the need to monitor these substances in surface, drinking and ground waters, at levels stipulated by EC and other legislation; and, a desire for methods to achieve this end that are more rapid and less costly than the traditional laboratory techniques such as GCMS. These aims have been met in part through the recent availability of immunoassay-based (ELISA) test kits from several manufacturers but whilst these offer a more rapid analytical capability, they are, by their very nature, less "user friendly" than true sensors.

Research aims to yield several classes of device:

- biosensors for use in on-line instruments for continuous and quasi-continuous field monitoring;

- biosensors and sensor systems for laboratory uses;
- disposable and re-useable biosensors for use in field instruments.

Examples are illustrated in the following sub-sections.

4.2.2 Re-useable resonant immunosensors

A team from Hewlett Packard's Palo Alto laboratory have developed a prototype biosensor for atrazine, based on immunoaffinity and a surface transverse wave quartz transducer operating at 250 MHz, coated with a polyclonal atrazine antibody. Atrazine binds with the antibody and mass-loads the transducer. Atrazine has been detected over the range 0.06 ppb to 10 ppm and the response time was less than 3 minutes. The sensor can be chemically reactivated, is therefore re-useable, and has been tested for over 100 determinations (Tom-Moy 1995). Several other groups are investigating various classes of antibody coated resonator sensors with equally encouraging results.

4.2.3 On-line FIA systems

The German GBF research institute has undertaken much research aimed at quasi-continuous, on-line pesticide determinations. Biosensors based on immunoassay and other techniques discussed in this section have been incorporated into FIA systems. Examples of analytes, the detection limits achieved and sensing principles used in this work include:

- atrazine and propazine: 0.02 and 0.002 ppb respectively
(polyclonal antibody immunoassay with fluorimetric detection);
- chlorfenvinphos and malaoxon: 1.0 and 9 ppb respectively
(cholinesterase inhibition with photometric detection);
- diuron: 5.0 ppb
(cyanobacterial oxygen production measured with Clark electrode, see 4.2.5, below).

Several other groups are involved with biosensor-based FIA systems and detecting the herbicide 2,4-D in this manner is the target of work at the University of Munster. The sensing element is a fused silica capillary with reagents immobilised on its inner surface. By using enzyme-labelled, high affinity monoclonal antibodies in a PC controlled FIA system, a detection limit of 0.1 ppb was achieved in 12 minutes. The range is 0.1 to 50 ppb (Trau et al 1995).

4.2.4 Optical immunosensors

The pan-European BIOPTICAS project serves as an example of research involving a combination of immunoassay and advanced optical sensing. The project involves six partners from three European countries, with a strong UK input from the Universities of Southampton and Liverpool and project management by GEC-Marconi. The approach is to immobilise pesticide-specific antibodies on an integrated optical sensor which detects surface binding by Mach-Zehnder interferometry or surface plasmon resonance. The target analytes are simazine, atrazine and isoproturon. Some detection limits so far achieved are: simazine - 0.5 ppb and 1.6 ppb (SPR and M-ZI techniques, respectively), and isoproturon - 0.05 ppb. Simazine has been detected at 0.2 ppb by spectral interferometry (see Wilkinson 1995, and Harris et al 1995).

A good example of a FO immunosensor is represented by research at the GBF. This involves binding a fluorescently labelled monoclonal antibody onto the core of an optical fibre. The analyte was detected by measuring the decrease in fluorescence coupled into the fibre by the evanescent wave, caused by the inhibition of the antibody binding to the fibre. The sensor has detected terbutryn with a resolution of 0.1 ppb (Bier et al 1992).

4.2.5 Live cell biosensors

Attempts to harness the toxic effects of pesticides on living systems and exploit them in biosensors is an obvious objective and many classes of micro-organism have been studied in this context for measuring BOD and toxicity. Recent work at the GBF is representative of that aimed at the

determination of herbicides. A biosensor has been fabricated that features an illuminated colony of cyanobacteria, immobilised on an oxygen electrode. The analyte inhibits the cyanobacterial photosynthesis, leading to a reduction in the liberated oxygen. These types of biosensor are less sensitive than those based on IA but the GBF device has successfully detected diuron at 5 ppb. However, it is most probable that live cell effects will be of greater use in measuring BOD and toxicity, than in the determination of pesticides.

An example of an approach involving cell components (rather than intact, living cells) is illustrated by recent research at the German Kernforschungszentrum Karlsruhe. This involves measuring the fluorescence emitted by immobilised chloroplasts, when illuminated by a laser at 635 nm. The sensor has detected photosystem II herbicides, such as triazines, at 0.5 ppb (Merz et al 1994).

4.2.6 Biosensors based on enzyme inhibition

The inhibition of cholinesterase type enzyme reactions by OP and carbamate compounds is well documented and has been exploited commercially for some years, eg. in Thorn's military NIAD nerve agent detector. Many groups have developed prototype biosensors based on this approach and work at the University of Perpignan is typical. Here two enzymes, acetylcholinesterase and choline oxidase have been used to initiate the reaction whereby the former converts acetylcholine to choline which reacts with the choline oxidase to liberate hydrogen peroxide. This is determined amperometrically by a platinum working electrode and a Ag/AgCl reference. The pesticide inhibits the first reaction and a prototype sensor has detected paraoxon at 10 nM concentrations (Marty, 1992).

Work at the University of the West of England is developing disposable thick-film sensors based on similar principles. Here, thiocholine is produced by a reaction of acetylcholinesterase on acetylthiocholine. The pesticide-induced reduction of activity is measured as a decrease in the oxidation current between the thiocholine and cobalt phthalocyanine on the electrode. Paraoxon and dichlorvos levels of 6.5×10^{-7} and 1.7×10^{-6}

mol/l have been detected (Hartley and Hart 1994). Some preliminary work involving non-immobilised reagents has detected low concentrations of sulphur-containing OP pesticides such as malathion and parathion; the former at a concentration of 4.07×10^{-8} mol/l.

4.3 BOD

4.3.1 General commentary

As illustrated well by commercially available products (see Table 3.1), the rapid determination of BOD has been a major objective of biosensor developments, despite the fact that BOD-5 remains the standard determinand and that measurements of "short-term" BOD may or may not correlate with this. It should be noted, however, that the Japanese environmental authorities now accept short-term BOD as a satisfactory measure of an effluent's ability to deplete oxygen.

Live cell technology is the dominant theme of prevailing work but since the commercialisation of products based on this approach, research has declined noticeably. Most publications pre-date 1990 but more recent work by researchers at the German company Bruno Lange is representative. A BOD sensor based on a colony of *Trichosporon cutaneum*, immobilised in polyvinyl alcohol was reported at "Biosensors 92" (Riedel and Uthemann 1992). This operates amperometrically and exhibited a linear response up to BOD concentrations of 2000 ppm in less than 5 minutes.

Note that Ecossensors Ltd. is involved in the commercial development of disposable biosensors for BOD (see Table 3.2, above) and both Cambridge and Cranfield Universities claim varying degrees of expertise in this field.

4.4 Toxicity

4.4.1 Research overview

In the environmental context the term toxicity may relate either to the toxicity of an effluent on the fauna or flora of a water course, or on activated sludge in a water treatment plant. Almost all biosensors developments are based on live cell technology, although some groups are investigating the possibility of incorporating the enhanced chemiluminescence reactions into biosensors. In the former instance, both optical and electrochemical techniques are under study although in recent years, optical methods appear to have attracted the greatest interest.

It should be borne in mind that, to date, no truly broad-band toxicity sensors have been developed. At the risk of confusing an already complex situation, it may be argued that several of the following are more realistically viewed as pesticide/herbicide or metals sensors, and equally, of course, some sensors described in those terms are, in effect, toxicity sensors.

4.4.2 Optical live cell biosensors

The Microtox test is an example of the use of luminescent micro-organisms to determine toxicity and several groups have applied this approach to true biosensors. A Japanese team (Suzuli et al 1992) reported a biosensor based on recombinant DNA technology in which Firefly luciferase genes were introduced into *E. coli*. The recombinant *E. coli* cells were immobilised on magnetic beads. Toxic compounds inhibit the resultant light from a solution containing the sample, the bacteria and a set quantity of luciferin, within a flow-through sensor system. Light reductions of 12% were achieved with samples containing 0.1 ppm metoxuron. Measurable light reductions resulted also from 50 ppb levels of isoproturon, ioxynil and propanil.

A variant on the conventional live cell approach has been demonstrated by workers from Liverpool John Moores and Poznan Universities (Grabowski et al 1995). This aims to determine the toxicity of metals to activated sludge

and combines optical fibres with intra-cellular enzyme activity. Toxicity is determined by measuring the rate of fluorescein diacetate (FDA) hydrolysis. FDA does not fluoresce when dissolved in water but in the presence of living cells, it diffuses into the cell's cytoplasm, where esterase enzymes hydrolyse the FDA into fluorescein which fluoresces when illuminated. Metals and other toxic pollutants inhibit the cells' metabolic activity and thus influence the rate of increase of fluorescence caused by the metabolism of the FDA. The fluorescein released by the cellular hydrolysis fluoresces at 515 - 520 nm and the light is captured by an optical fibre and measured with a photodetector. The light's intensity is monitored as a function of time and the gradient of this curve equates to the toxicity of the sample.

Work at PA Consulting (UK) has investigated UV spectrophotometry as a means of characterising toxicity induced changes in cellular activity (Bains 1992). E. coli cells were incorporated into an agarose membrane which was exposed to the sample in a flow-through arrangement. A significant change in the cells' optical density at 200 nm occurred when the membrane was exposed to toxic species such as sodium azide. Various mechanisms have been proposed to explain this effect.

Note also the collaborative development for an on-line version of Microtox, see Table 3.2, above.

4.4.3 Non-optical live cell techniques

The work at Luton University, some having been supported by the NRA, involving mediated amperometry, is representative. The principle is to immobilise living cells on an electrode, and through the use of a mediator such as potassium ferricyanide, measure the toxicity-induced reduction in current at the electrode, caused by a fall in the nutrient consumption of the organisms. Plant or animal cells can be used, although the former require a light source. Neither have exhibited a particularly broad-band or repeatable response.

4.5 Phenols

4.5.1 Research overview

Phenols can create significant water pollution in areas in close proximity to industries producing or using these compounds, or where cresols are used to treat mastitis on cows. Pentachlorophenol is, of course, a Red List species.

Biosensors for phenols invariably involve the oxidation of the analyte by one or more enzymes, such as phenoloxidase and tyrosinase, and many groups are investigating this approach. Some examples of prevailing research are considered below.

4.5.2 Enzyme-based biosensors

The work by Turner at Cranfield is representative. Here, the enzyme tyrosinase has been incorporated into a disposable, thick-film sensor. The enzyme reacts with the analyte and the results of the oxidation are determined amperometrically. Research is at the working prototype stage and 25 nanomolar concentrations (2.5 ppb) of various species have been detected. Work at the UWE has demonstrated the use of glucose oxidase and pyruvate oxidase to detect pentachlorophenol and phenol with lactate oxidase and lactate dehydrogenase (Cowell et al 1994). The reactions have been incorporated into a prototype multi-analyte enzyme array.

Polyphenol oxidase has been used to determine pyrogallol, catechol, phenol and p-cresol in the 10^{-5} M range by a group of Brazilian workers and the sensors have been used to determine these compounds in industrial wastewater (Fatibello-Filho 1994). The sensors responded in 1-4 minutes and had useful lifetimes of over two weeks.

Gorton and co-workers from the Swedish University of Lund have undertaken much work on this topic. For example, an EC-funded project (number EV5V-CT92-0109, see subsection 5.3.2, below) between Lund and workers from various Spanish and Dutch establishments is investigating the use of tyrosinase and related enzymes in various biosensor configurations to

determine 50 phenolic compounds, taken from the EEC and US EPA lists (Marko-Varga et al 1994). Various phenolic species have been detected at ppb levels (Dominguez and Ortega 1994).

4.6 Gases

4.6.1 Introduction

Gas detection plays a major role in environmental protection and most sensors are located at the point of discharge (stack gas analysers) or in the external environment (fixed ambient air quality monitors). Smaller numbers of instruments are used to scan the atmosphere around gas sources such as landfill sites, petrochemical plants and gas storage facilities.

Most of these applications are satisfied by a battery of optical sensing techniques that are selective, sensitive, stable, reliable and reasonably robust. Examples include IR absorption, UV absorption, UV fluorescence, chemiluminescence and LIDAR. Thus, prospects for biosensor-based measurements are seen as extremely limited but it may be that, in the event of species that cannot be determined by these or any other existing methods, biosensors could play a role. Dioxins may fall within this category.

There is, as yet, little research into gas responsive biosensors but the following subsections provide an insight into current work. Note also the British Gas biosensor for monitoring MEG in air (see Table 3.1 and Appendix B).

4.6.2 Enzyme-based gas biosensors

Cranfield is involved in an EC funded collaboration with City Technology, the UK electrochemical gas sensor manufacturer. Work is investigating the role of generic, enzyme-based methods to determine species such as SO₂, phenolic vapours and CH₄. As with most other enzyme approaches, detection is amperometric and the microelectrode sensors are fabricated by thick-film

technology. Work is still at an early stage but results to date suggest that this approach has real prospects; phenol vapour has been detected at around 1.6 ppm (Karayannis et al 1994). Applications, however, are more likely to be found in occupational health and safety rather than environmental monitoring areas, at least in the short term.

4.6.3 Biosensors for airborne biochemicals

Research involving the Universities of Sunderland and Teeside has been investigating for some time the use of immuno-based biosensors to detect airborne enzymes such as protease. These occur in various working environments such as detergent manufacture and pose a threat to health if inhaled. Various antibodies have been immobilised on the surfaces of substrates with interdigitated electrodes and protease levels of 0.05 to 50 mg/ml have been achieved. This work is reviewed in detail by Koochaki et al, 1995, but as with the above, applications are anticipated within the workplace rather than in the external environment.

4.6.4 Other concepts

This sub-section considers briefly various concepts and principles that might, in the longer term, offer prospects in the environmental monitoring context.

One is the use of evanescent wave interactions in conjunction with FO or IO gas sensors. Many groups have coated optical fibres and IO structures with non-biological gas sensitive reagents which react with the analyte via the waveguide's evanescent wave. Biological agents could be used as the coatings but as yet, little work is reported. Point and distributed FOSs based on this concept might be able to determine various VOCs etc.

Rawson and co-workers (University of Luton) have undertaken some preliminary work on the use of live cell-based biosensors to determine air toxicity. The work was not successful and Capteur Ltd., one of the commercial parties, withdrew from the project. Other groups are working on similar schemes but any prospects are certainly of a longer term nature.

Immunoassays could selectively and sensitively determine species such as dioxins and furans, and test kits exist for the determination of these in water and soil samples. However, the very low levels of these compounds in air samples, eg. incinerator emissions, (typically in the ng/m^3 range), would obviously necessitate the use of preconcentration techniques.

4.7 Other analytes

As illustrated by Table 4.1, prototype biosensors have been developed for many analytes of environmental interest, in addition to those considered above. The following sub-section provides brief details of work on biosensors for metals, PCBs, micro-organisms and silage liquor.

4.7.1 Metals

Several groups have developed metal responsive biosensors, based on a variety of techniques such as monitoring the toxic responses of living cells (see above), enzyme reactions and so forth.

A collaborative pan-European project involving workers at Cranfield University and elsewhere is investigating amperometric biosensors based on the change in redox response of a ligand when it complexes with the metal. Iron and copper have been determined in this manner by using desferrioxamine and bis-cyclohexanone-oxalyldihydrazone as the complexing agents, respectively (Bannister et al 1994). A Japanese group has detected Zn(II) ions with a calorimetric biosensor which uses beads coated with the enzyme thermolysin in a FIA system. Zn(II) ions can be determined in the concentration range 0.01 to 1.0 mM (Sato 1995). Work at the University of Cambridge on behalf of BNF plc is investigating enzyme based methods to detect heavy metals such as Hg etc. in aqueous effluents (see 5.6.4, below). There are numerous other examples in the recent literature.

Whilst biosensors should not be ruled out as offering a longer term metal sensing option, in view of the comparative instability of most biological

agents, it seems more likely that, generally, the voltammetric techniques that are presently attracting much attention will be better suited to this task in the short term.

4.7.2 PCBs

Whilst a number of IA-based test kits have been launched recently that respond to PCBs, there is relatively little literature on biosensors for this group of analytes.

Work at the German Institute of Chemical and Biochemical Sensor Research (Munster), in collaboration with other European groups (see Table 5.2, below), aims to develop simple and inexpensive sensors and systems for PCBs in water. These include a FIA system for continuous monitoring and disposable sensors for monitoring PCBs in the field. A technique is being used whereby immobilised antigens will compete with the analyte and enzyme-labelled antibodies in a competitive ELISA scheme. Following the reaction, enzyme substrates are added which generate a product that is detected at a working electrode. Measuring electrodes and Ag/AgCl reference electrodes will be fabricated by the thick-film process (Mascini et al 1994).

Interestingly, some work by Slater at Birkbeck College has investigated the feasibility of using non-bio-based sensors to detect these compounds. This was funded by EA Technology on behalf of the UK power generation industries and involves screen-printed electrodes with a selective extractive coating that pre-concentrates the analyte, prior to its electrochemical detection. Low ppm levels have been detected and whilst these are likely to be higher than those normally found in water courses, prospects for this technology may exist within the waste regulation side of the forthcoming EA.

4.7.3 Micro-organisms

Several groups are investigating techniques to determine concentrations of micro-organisms in water. Indeed, a biosensor based product already exists for this, the Midas PRO, (see Table 3.1 and Appendix B).

Research at the Japanese Saitama Institute of Technology illustrates one aspect of current work. This involves the determination of viable cell numbers by the amplified detection of catechol which is produced in microbial co-metabolism by *E. coli*. The reaction is amplified by L-ascorbic acid and the sensor uses a tyrosinase (catechol oxidase) electrode whose output is proportional to *E. coli* concentrations over the range 10^3 to 10^5 cells/ml (Hasebe et al 1994).

Work involving research groups from Norway and Belgium and the NRA (see Table 5.2, below), involves the development of a technique to determine fecal coliforms in bathing water. This relies on the enzymatic hydrolysis of fluorogenic 4-methylumbelliferyl derivatives which yields fluorescent products. A prototype instrument is presently undergoing field trials with the NRA, and full project details are given by Nelis et al, 1994. An example of the potential role of silicon biosensor technology is illustrated by work at the University of Genoa, working with Omega SRL. The group has developed a prototype flow-through system for the on-line determination of micro-organisms in water. The sensor is a hydrogen ion (ie. pH) sensitive ISFET and operates by measuring living cell-induced acidification rates. It comprises a matched silicon nitride gate H^+ FET/Al gate FET pair, together with on-chip signal conditioning electronics. Changes ranging from around 0.003 to 0.15 pH were obtained for concentrations of between 10^5 and 10^7 *E. coli* cells/ml (Gambiaso et al 1995).

4.7.4 Silage liquor

There is very little in the literature on this analyte but Turner and co-workers (University of Cranfield) have undertaken some preliminary work involving biosensors to detect the presence of silage in water courses.

The problem is identifying a suitable, stable analyte as the silage indicator and following extensive screening, it was found that lactate, which is a persistent by-product, was suitable. The sensors detect this through a reaction with the enzyme lactate oxidase. Work to date has

demonstrated the validity of this approach and the sensors would be disposable and fabricated via thick-film printing technology.

This work was funded originally by Yorkshire Water, and although some further work is required to increase sensitivity, it is at a stage where commercialisation is a realistic proposition.

4.8 Soil analysis

The analysis of soil for pollutants such as PCBs, PAHs, dioxins, metals, pesticides, oils and other organics is a field of growing interest, particularly in the context of contaminated land investigation and remediation. Interest is strongest in the US, where several ELISA-based field test kits that respond to these species have been developed for these uses. Also, several non-invasive techniques such as prompt gamma neutron activation analysis (Bogue 1995a) and laser-induced breakdown spectroscopy (Bogue 1995b) are being developed for the real-time, in-situ analysis of soil contaminants such as metals and oils etc.

The paucity of reported work in the open literature probably reflects the perceived obscurity of this application within the academic community rather than any fundamental technological restraints, and at least one project is in progress. This involves the Universities of Cranfield and Munster and the German company Cell Diagnostica GmbH (see Table 3.2, above), and aims to develop IA-based electrochemical biosensors to determine the pesticide 2,4-D in soil, with minimal sample preparation.

Soil is a complex matrix and extraction techniques are invariably required before any sensor, bio-based or other, can be applied. However, the extraction procedures adopted by EnSys (US manufacturers of ELISA soil test kits) are very simple and take only around five minutes, and it follows that the same extraction procedures could be applied to biosensors.

The ultimate role of biosensors in this context remains unclear but prospects would appear to be strong in the context of solid waste screening and the analysis of soil around landfill sites by the WRAs.

5. RESEARCH GROUPS AND PROJECTS

5.1 Introduction

As the previous sections illustrate, environmental biosensor R&D activities are spread widely throughout Western Europe and elsewhere. The UK is fortunate in having a particularly strong community of academic groups and at least two are of international standing (Cranfield, Cambridge). The UK's national effort in (all) biosensor technology was appraised recently by the author on behalf of the DTI (Bogue 1994), and was rated as "excellent" and deemed to be a significant national asset. Details of the activities of most UK groups are given in the same source.

Several very strong groups are located in Germany, with lesser numbers in France, Italy, Scandinavia and other European countries. Japan has at least one undisputed centre of excellence, Karube's group (see Table 5.1), whilst the US is relatively weak. Much US research remains focussed on clinical applications, probably reflecting the large potential domestic market for diagnostic products, although some university groups are now working in the environmental area. Amongst the more noteworthy are Cincinnati, Maryland and Tufts Universities.

However, in the context of groups with whom the NRA/Agency might consider collaborating, it is the author's view that expertise within European institutions, mostly in the UK and Germany, is as strong as that anywhere in the world.

5.2 Academic research groups

A number of key academic research groups are listed in Table 5.1. This list should be viewed as representative only and the exclusion of any group should not be seen as implying its lack of relevance or importance. However, the list includes many of the groups that could be potential partners in any future collaborations.

TABLE 5.1 - Some important environmental biosensor research groups

<u>Institution</u>	<u>Key workers</u>	<u>Activities</u>
<u>UK</u>		
University of Cranfield	Turner	Very broadly based, key group
University of Cambridge	Lowe	Very broadly based, key group
University of Luton	Rawson	Live cell technologies
UWE	Cowell	Enzyme sensors and arrays
<u>Germany</u>		
University of Tubingen	Gauglitz	Optical immunosensors etc.
Westfalische Wilhelms University/ICB	Cammann	FIA systems for pesticides etc.
GBF, Munster	Schmid	FIAs and FOSs for pesticides etc.
Institute of Radio-chemistry, Karlsruhe	Ache Reichert	Immunosensors for pesticides
University of Potsdam	Scheller	Broadly based, strong on BOD
<u>Elsewhere in Europe</u>		
University of Lund (SWE)	Gorton	Enzyme sensors for phenols etc.
University of Perpignan (F)	Marty	Enzyme sensors for pesticides
La Sapienze University (I)	Campanella	FIA systems for pesticides etc.
<u>Japan</u>		
University of Tokyo	Karube	Very broadly based, key group

5.3 EU environmental biosensor projects

5.3.1 Background

A number of collaborative, pan-European projects are underway which aim to yield biosensors and biosensor-based products for a variety of environmental monitoring applications.

These are funded by the European Commission, under the auspices of DG-XII, and form part of the Environment and Climate programme. Calls for proposals for new projects are issued each year and up to three or four may be accepted annually. The yearly EC budget is around ECU 2 million. Seminars are held each year to review and present progress and the next will be in Barcelona in February 1996.

5.3.2 Project details

Summary details of current projects are listed in Table 5.2.

TABLE 5.2 - Environmental biosensor research under DG-XII

<u>Project Title/number</u>	<u>Institutions</u>
Development of on-stream biosensors for pesticide detection EV5V-CT92-0104	Kings College (UK), University of Potsdam (D), Technical University of Berlin (D), Institute of Chemical Physics and Biophysics (Estonia)
Development of new biosensors and improvement of existing sensors by means of a 'smart sensor' concept - application to aquatic media EV5V-CT93-0245	University of Luxembourg (Lux), University of Perpignan (F), Centre for Public Research (Lux), University of Aarhus (Den), University of Barcelona (Spain)
The development of micro-biosensors for monitoring hazardous gases in the environment EV5V-CT92-0077	Cranfield University (UK), City Technology Ltd. (UK), University of Ioannina (Greece)

Continued overleaf

Table 5.2, continued

<p>Biosensor developments for environmental analysis of phenols and related compounds EV5V-CT92-0109</p>	<p>University of Lund (Sw), University of Alcala de Henares (Spain), CSIC (Spain), Leiden University (Neth)</p>
<p>Optical biosensor techniques for monitoring organic pollutants in the aquatic environment EV5V-CT92-0067</p>	<p>GEC Marconi and Hirst Research Centre (UK), University of Liverpool (UK), University of Tübingen (D), University of Southampton (UK), IOT (D), Bureau de Recherches Géologiques et Minières (F)</p>
<p>Development and validation of s-triazine- and pyrethroid-specific immunosensors EV5V-CT94-0358</p>	<p>University of Stuttgart (D), Technical University of Munich (D), ENEA (I)</p>
<p>Immunobiosensor developments for monitoring of pesticides and their transformation products EV5V-CT93-0354</p>	<p>University of Alcala de Henares (Spain), Lund University (Swe), CSIC (Spain), TNO (Neth), Leiden University (Neth)</p>
<p>Development of a biosensor for monitoring of bacteria in water EV5V-CT93-0345</p>	<p>University of Gent (Belg), NRA (UK), University of Trondheim (Nor), Aquateam A/S (Nor)</p>
<p>A portable amperometric biosensor system for the monitoring of heavy metal ions, bacteria, pesticides in water EV5V-CT94-0357</p>	<p>University of Cranfield (UK), University of Padova (I), Technical University of Munich (D), University of Barcelona (Spain)</p>
<p>Monitoring of polychlorinated biphenyls (PCBs) in ground water through an immunochemical biosensor with electrochemical detection EV5V-CT94-0407</p>	<p>University of Firenze (I), ENEL-CRC (I), University of Barcelona (Spain) Institut für Chemo- und Biosensorik Münster (D)</p>

5.4 The US EPA programme

5.4.1 Background

The US EPA has a large and highly active biosensor R&D programme which is investigating many applications and technologies, most through collaborations with academic institutions. Applications include laboratory screening, field screening, continuous monitoring and in-situ field monitoring. Some of the analytes under study include:

- phenols;
- organic peroxides;
- triazines;
- PCBs;
- various aromatics;
- organonitriles;
- carcinogens that bind to DNA;
- TNT;
- micro-organisms.

Technologies and sensing techniques include:

- immunoassays;
- fibre optic biosensors;
- FIA systems;
- enzyme electrodes;
- live cell biosensors;
- evanescent wave and fluorescent effects.

The EPA is a member of the NIST (National Institute for Standards and Technology) CAB project (Consortium on Advanced Biosensors) which aims to resolve the technical problems inhibiting the more widespread uses of biosensors.

5.4.2 Project details

Summary details of a number of EPA supported projects are listed in Table 5.3 and a fuller review of this work have been given recently by Rogers (1995).

TABLE 5.3 - Environmental biosensor research by the US EPA

<u>Topic</u>	<u>Establishments involved</u>
Amperometric enzyme electrodes for phenolics and organic peroxides	New Mexico State University
Capillary enzyme immunoassays with electrochemical detection for atrazine	University of Cincinnati
Fibre optic biosensors for pesticides and PCBs	University of Maryland
Fibre optic immunosensors for atrazine	Tufts University
Fibre optic fluorescent immunosensors for 2,4-D etc.	EPA labs, Las Vegas
Fluorimetric biosensors for carcinogens that bind to DNA	National Institute for Standards and Technology (NIST)
Fibre optic biosensors for detecting bacteria	Naval Research Laboratory, Washington DC
Electrochemical enzyme biosensors for organonitriles (eg. benzonitrile)	Tufts University

5.5 The NRA programme

The NRA has undertaken some work involving biochemical measuring techniques (rather than biosensors), for example, the ECLOX work and investigations into the use of IA-based test kits, but has pursued very few biosensor R&D projects. It has no formalised programme of involvement with this

technology. HMIP has an interest in, but no current involvement with, biosensors.

Summary details of the two NRA projects known to the author are given in Table 5.4, which illustrates the programme's weakness when compared to that of the US EPA.

TABLE 5.4 - Biosensor research by the NRA

<u>Topic</u>	<u>Establishments involved</u>
Live cell biosensors for water toxicity (BIOTOX, broad spectrum biosensors)	University of Luton, WRc
Biosensors for monitoring bacteria in water (EU project, see Table 5.2) 1994-1996	University of Gent, Norwegian Water Technology Centre A/S, University of Trondheim

5.6 Other UK projects

This section reviews various other UK organisations' involvements with biosensors, with the objective of identifying common interests and potential partners for collaboration with the NRA/Agency.

5.6.1 MAFF and the food industry

The MAFF and other parts of the food industry are involved with the development of test kits and analytical techniques based on immunoassay, eg. to determine food quality and detect pathogens and contaminants such as herbicide residues. Amersham is collaborating with the University of Nottingham and various major food producers and retailers on a project which aims to determine pathogens with a bioluminescent technique, and IFR

Norwich, Cortecs Diagnostics and other partners are developing monoclonal antibody based methods to determine wheat gluten quality.

However, there is very little evidence of the development of true biosensors within these industries, although the University of Cambridge is developing an enzyme-based biosensor for ethanol and work by Professor O'Gara at the Department of Food Microbiology at University College, Cork, is attempting to develop an acoustic biosensor featuring immobilised antibodies to detect food spoilage and/or pathogenic bacteria. The IFR (Norwich) is planning to work with Rawson's group (Luton University) on the development of immunosensors for pesticide residues and expressed an interest in collaborating with the NRA on this or any other projects where there might be common interests. The contact is Dr G Wyatt (tel. 01603-255000).

5.6.2 The NERC

The only biosensor related activities within the NERC laboratories are underway at the Plymouth Marine Laboratory (PML). Nelson and Lowe are heavily involved in, and have much expertise with, biological membrane and electrode technology. They have studied the effects of toxic compounds on cellular membranes and developed synthetic membranes that could feature immobilised active reagents such as antibodies, and be used in conjunction with various electrodes. Analytes of interest include pesticides and various aromatics. The group is, however, short of funds and expressed a keen interest in collaborating with the NRA/Agency on any biosensor topics of mutual interest. The contacts are Dr Andrew Nelson and Dr David Lowe (tel. 01752-222772).

5.6.3 The water supply industry

Whilst prospects to collaborate with any of the UK's ten major water supply companies are probably slim, it is worth mentioning briefly some of their recent biosensor research.

Yorkshire Water probably has the largest programme and has supported much research in this field. Some examples include: multi-biosensor arrays for pollution monitoring (with the University of the West of England); phenol detection by bioluminescence inhibition (University of Leeds); phenol detection with enzymes (University of Cranfield); silage effluent detection by lactate monitoring (University of Cranfield); heavy metal detection with genetically engineered micro-organisms (University of Aberdeen); respiration monitoring with live cell technology (University of Cambridge); on-line toxicity monitoring (Siemens Plessey, UMIST and Microbics); monoclonal antibody based methods for detecting Cryptosporidium; dielectrophoresis to determine bacterial numbers and species; and ATP bioluminescence for toxicity.

Anglian water is working closely with Lowe's group at the University of Cambridge and has a shareholding in Environmental Sensors Ltd., Lowe's company that was set-up to commercialise biosensor products. Severn Trent Water has some involvement with biosensors and it is highly probable that several of the other water plcs are also actively investigating this technology.

The key point to note is that the water companies, ie. the regulated, are investigating the role of biosensor technology far more actively than are the regulators - the NRA and HMIP.

5.6.4 Other projects

Two specific UK projects warrant brief mention here. The University of Cambridge (Lowe) is collaborating with BNF plc, via a LINK project, on the development of a low cost, enzyme-based optical biosensor. This is aimed at the determination of nano-molar concentrations of mercury and other heavy metals in aqueous effluents. In a second LINK project, Bookham Technology Ltd. is working with Cranfield University (Turner) on the commercial development of a family of low cost optical interferometric biosensors based on silicon waveguide technology. These are aimed at analytes such as metals and pesticides etc., as well as various non-environmental species (Newman et al 1995).

In both of these instances the work appears to be of relevance to the interests of the NRA/Agency, and discussions with the parties involved are recommended. There is certainly scope for direct collaboration with Bookham.

5.7 Projects by other European environmental bodies

5.7.1 Overview

Despite the number of EC funded environmental biosensor projects and the activities of the US EPA, there is minimal evidence of programmes being funded or supported by continental European environmental protection agencies. As illustrated by Table 5.2, the only such body involved in the current suite of EC-funded programmes is the NRA. This lack of activity reflects, in part, that in many countries, such as Germany and France, there is no single body with overall responsibility for environmental protection.

5.7.2 Status of activities in selected countries

The Irish EPA is aware of biosensors but the agency has no R&D programme or clear strategy on this topic, partly reflecting an acute lack of funding. No research is presently being supported in any areas. The prevailing approach is to await the availability of commercial biosensor products.

The situation in Denmark is little different. The Danish EPA has some interest in biosensors but is not, apparently, undertaking any work at the present time. The emphasis appears to be on investigating the impact of pollutants on human health and the aquatic environment, rather than developing detection techniques. This is certainly the case within the pesticides division. A project with the University of Odense is using ELISA techniques to determine the uptake of complex phenols by fish but the emphasis is on the fish's metabolism rather than on the measuring technique. There does not appear to be any clear strategy on biosensors.

In the Netherlands a study into the role of biosensors and other sensing and analytical technologies has just been completed on behalf of STOWA (the Foundation for Applied Water Research). This was undertaken by the International Centre for Water Studies (ICWS, Amsterdam) and was in response to a perceived need by the Dutch water boards for rapid, cost-effective screening methods to determine many of the analytes governing water quality. Four technologies were investigated: biosensors; immunoassay methods; bioassays; and HPLC fingerprinting. The conclusion reached was that biosensors have not yet reached the point where useable products are available, but two other techniques offer strong, shorter term prospects: immunoassays and HPLC fingerprinting. These will be developed further and the work co-ordinated by the ICWS. The contact at STOWA, Dr S Klapwijk (tel. 0031-30-232-1191), expressed the view that collaborating with the NRA in the future could be a possibility. RIZA has concluded that, although of great potential interest, biosensors are still some way from being useable and the strategy is to await commercial developments. There is, however, a strong interest in biomonitoring techniques such as fish- and Daphnia-based pollution monitors, which are now used on two major Dutch rivers.

Germany's water supply and environmental monitoring organisations are very conservative, perhaps even more so than their UK counterparts. Although they frequently request data on progress on biosensor developments from the GBF and elsewhere, none of the various regional environmental protection bodies are known to be actively involved in any R&D. The GBF is, perhaps, Germany's leading biosensor research institute yet none of its work is supported by any environmental agencies. However, its work on on-line FIA systems for pesticide determination is now being commercialised by a medium-sized German company, in response to a request for an on-line method from one of the regional water companies in the Lander of North Rhine Westfalia. Prototypes for evaluation are expected in early 1996. There is much strong biosensor research at several German universities but there is no evidence of support from any environmental protection organisations. There are no formal mechanisms for technology transfer and potential users seem to be less involved with collaborative R&D than in the UK.

Interestingly, in the UK, the Netherlands and Germany, it is the water supply companies, rather than their regulators, who are making the running with this technology. Furthermore, the recently launched French on-line pesticide analyser (see Table 3.1, above and Appendix B) has been developed by a company, SERES SA, which is owned by a water utility.

6. CAPABILITIES AND APPLICATIONS

6.1 Introduction

Previous sections provide a reasonably detailed insight into the nature of available and forthcoming products, and of prevailing research. To recap briefly, available biosensor-based products, and those whose launch is imminent, can or will offer the ability to:

- 1) determine BOD rapidly in the laboratory from 2 mg/l upwards (no upper limit with sample dilution);
- 2) determine numbers of viable bacteria in water from around $10^5/10^6$ cells/ml upwards (with dilution), in the lab and field;
- 3) determine triazine pesticides on-line, over the range 0.05-1.00 ppb;
- 4) determine water/waste toxicity on-line.

Looking perhaps one to three years into the future, products known to be under commercial development are expected to further increase these capabilities, as below.

- 1) determine low concentrations of pesticides quickly and cost-effectively in the field. (water and soil);
- 2) determine toxicity in the field;
- 3) determine BOD rapidly in the field;
- 4) determine certain heavy metals in the field and in industrial effluents;
- 5) determine a number of toxic gas species.

6.2 Future capabilities

6.2.1 Specifics

To formulate a realistic strategy the NRA/Agency needs an insight into what may be expected from biosensors in the longer term, say, during the next five years. The following are seen as probable and realistic, although specialised products such as 4), below, may only emerge from user-supported developments. Equally, of course, other more specialised and user-specific capabilities could emerge from any such collaborations.

- 1) Families of disposable biosensors for the rapid and selective field determination of several specific pesticides and pesticide groups, at low/sub-ppb concentrations.
- 2) Similar biosensors for the field determination of phenols at ppb concentrations.
- 3) Field sensors to determine certain toxic metals (Hg, Cd etc.).
- 4) Disposable field sensors to detect silage liquor.
- 5) Improved lab and field instruments to determine various classes of micro-organisms in water, eg. fecal coliforms, total coliforms, fecal streptococci etc.
- 6) On-line analysers to determine micro-organisms in water/waste.
- 7) Improved on-line analysers for various specific pesticides and pesticide groups, at low/sub-ppb concentrations.
- 8) On-line analysers for other species such as phenols, etc.
- 9) Biosensor-based laboratory instruments to rapidly and simply determine complex organics such as PCBs, PCPs, dioxins, pesticides etc.

6.2.2 Benefits and limitations

It is important to understand the benefits that such developments may offer the NRA/Agency, and their limitations.

In terms of their broad capabilities, biosensors are no different from other classes of sensor, thus, biosensor-based field and on-line instruments will confer the same advantages as their conventional counterparts. Where they score over these is their ability to determine analytes in the field and on-line where formerly, determinations were only possible in the laboratory. Such analytes include pesticides and pesticide groups, phenols, BOD, toxicity, silage and micro-organisms, and perhaps in the longer term, certain metals and some of the other species listed in Table 4.2. Additionally, biosensors will be able to perform screening functions in the laboratory.

Reiterating the generic benefits of the sensor-based approach to environmental monitoring, field instruments may generate valid data in their own right in some applications and therefore confer the advantages of greatly reduced time in acquiring results and probably lower testing cost. Where positive samples need to be verified analytically in the laboratory, cost may be reduced by the near elimination of testing negative samples. Field instruments also allow certain pollutants to be identified and traced to source. On-line instruments offer the clear benefit of providing a far greater degree of surveillance and control of polluting discharges than sampling and lab analysis. Their widespread use in liquid effluent and stack gas emission monitoring is testimony to this.

Against these benefits are a number of limitations, most of which are generic to all classes of biosensors, namely:

- biosensors are likely, in the short term at least, to be less rugged than many of their conventional counterparts;
- shelf lives are likely to be shorter;

- on-line instruments are likely to suffer from many of the limitations that plague conventional on-line chemical analysers (eg. limited periods of unattended running, high ownership costs and often poor field reliability);
- in most instances, biosensors, like many other molecular sensors, are likely to suffer from certain cross-sensitivities, limitations in accuracy and resolution, and long term instabilities;
- many biosensors exploit several reactions and require the addition of defined quantities of reagents at set times during the overall measurement cycle;
- response times are likely to be longer than those of conventional sensors, due to the nature and number of reactions involved.

However, much biosensor research is addressing practical issues and aims to overcome, or at least minimise, many of these limitations. For example, with regard to stability, the enzyme glucose oxidase, immobilised in a polymer matrix, has been shown to remain active for 20 months, and disposability is seen as a means of overcoming lifetime and stability problems. It also eliminates the need for reversible reactions which expands enormously the design possibilities. Low cost fabrication technologies such as thick-film and ink-jet printing are expected to facilitate disposability and integrated sample handling and conditioning technologies such as printed liquidic circuits, microfluidics and various microengineering techniques may well eliminate the need for the manual addition of reagents and timing of the various stages involved in the overall transduction process. FIA systems also overcome this problem and are well suited to quasi-continuous on-line monitoring applications and laboratory uses.

As with all other classes of sensors, many of the initial limitations of biosensors will be overcome as the technology matures.

6.3 Applications within the NRA/Agency

A number of potential applications for biosensors within the NRA, WRAs and the forthcoming EA are proposed in this section. These are based on the capabilities anticipated from biosensors over the next five years (see above) and the perceived needs of the NRA. The needs for field measurements by the NRA and the WRAs, identified during a recent NRA R&D project into the role of field test kits, (Bogue 1994), are also taken into account.

6.3.1 Laboratory screening

Several laboratory applications are visualised. The majority relate to the rapid screening of water samples, prior to more rigorous analysis by conventional means. Within the present structure of the UK's environmental protection organisations, users would most probably be the NRA and the WRAs. Examples of such uses include the following.

- 1) Rapid screening of surface water and liquid effluent samples for BOD.
- 2) Rapid determinations of bacterial numbers in bathing and waste waters (an aim of the NRA's research project on this topic).
- 3) Rapid screening of surface water samples for pesticides.
- 4) Rapid screening of surface and waste water samples for PCBs, PCPs and, perhaps, dioxins.

Products are commercially available to undertake the first two functions, above, (see Table 3.1) and ELISA-based test kits are available for the last two.

6.3.2 Field water quality measurements

Portable instruments employing (probably disposable) biosensors could facilitate the rapid screening and quantitative/semi-quantitative measurement of several species in the field, where now, determination is only possible in the laboratory.

Applications range, potentially, from routine water and effluent quality monitoring to investigating polluting incidents, testing landfill leachates, tracing sources of pollution and protecting drinking water supply intakes. Measurements could be applied to rivers, lakes, effluent streams, landfill liquors and drinking water sources. Potential uses are summarised below.

- 1) Rapid field determination of pesticides in surface waters.
- 2) Rapid field determination of BOD in surface waters, waste waters and landfill leachates.
- 3) Field determination of phenols in surface waters and effluents.
- 4) Field determination of PCBs and similar in surface waters and effluents.
- 5) Field determination of water and effluent toxicity.
- 6) Detecting silage liquor.

As yet, no products are available to fulfill these tasks but as with the above, ELISA-based kits are available for the field measurement of pesticides and PCBs. At least two products exist which claim to yield surrogate field measurements of BOD, although these are based on the enhanced chemiluminescent reaction rather than true biosensors.

6.3.3 Air quality monitoring

It is unlikely that biosensors will play a role in air quality monitoring within the environmental context within the next five years. As noted above, most existing applications are presently satisfied by instruments using well proven and rugged optical sensing techniques and most gas responsive biosensor research is aimed at health and safety applications.

In the longer term biosensors may have a role in monitoring organic species that cannot be determined by such means, and could include dioxins and various VOCs. However, it is unlikely that biosensors will offer sufficient long term stability to be used in ambient AQ monitors, or be sufficiently rugged to monitor stack emissions. Applications might exist in portable instruments to, for instance, track emissions of organic species or locate their sources.

6.3.4 Solid and liquid waste analysis

The WRAs are involved in the analysis of a large number of compounds in both liquid and solid waste and presently employ a combination of field test kits, portable instruments and a limited amount of laboratory analysis. During the survey on the needs for field test kits (see above), requirements were voiced by the WRAs for rapid field methods to determine several species in industrial waste, where few or no realistic methods exist. These included:

- phenols;
- toxicity;
- PCBs;
- cyanide.

Biosensors have been reported that respond to all of these analytes and it would appear, therefore, that biosensor-based field instruments could contribute to meeting these requirements. The widespread use of ISEs etc. by the WRAs, and their limited field lives, supports strongly the view that disposable biosensors could be of considerable utility.

The analysis of solid waste is a more complex issue but as discussed above, with the development of appropriate extraction techniques, biosensors could play a role here too, but perhaps only in the somewhat longer term.

6.3.5 Fixed site and on-line water quality monitoring

Within the context of use by the environmental protection bodies, fixed site and on-line monitors are used to protect drinking water intakes, provide continuous surveillance of vulnerable water courses and spot sudden causes of pollution. On-line monitors are used widely by the industries discharging to water, whether regulated by the NRA or HMIP, and in some instances on-line monitoring is stipulated by the regulating authority.

The availability of biosensor-based on-line and fixed site monitors for analytes such as BOD, toxicity, pesticides and phenols would appear to expand usefully such capabilities. Their potential utility is clearly

recognised by the water supply and waste sectors, as evidenced by the developments supported by these industries in several countries.

6.4 Cost considerations

All of the above scenarios for biosensor deployment confer different potential benefits, whether operational, financial or both. However, it is recognised that the demonstration of economic benefits is most likely to stimulate serious interest in this technology.

Two potential uses are considered here: screening water samples in the field for BOD prior to conventional analysis, and applying the same approach to dioxin analysis. In the former instance, the most clear-cut benefits are operational and the economics are marginal, whilst in the second the dominant benefits are economic.

Consider the following, which relate to the NRA's present activities:

Number of BOD determinations/year:	c. 500,000
Laboratory cost of each:	c. £3.00
Number found to be negative:	c. 30%
Cost of each field determination:	c. £1.00 (estimate by potential manufacturer)

Based on the above the total cost now is c. £1.5 million. Screening all samples in the field would cost £0.5 million and if 30% were benign, the 350,000 positive samples would still need to be tested in the lab, at a cost of £1.05 million. Thus, the total cost of adopting this approach would be £1.55 million, or marginally more than the conventional approach.

Obviously, the figures used here are estimates only but they serve to illustrate that any economic benefits would be, at best, marginal. However, the operational benefits are more obvious: field screening would allow decisions to be made on the spot; serious pollution incidents could be

dealt with in real-time; and sources of pollution could be traced to source and hopefully rectified.

In the case of dioxins, there are clear economic benefits. Presently the NRA subcontracts its dioxin analysis at a cost of around £1000/sample and only a few tens of analyses (say 30) are undertaken annually. A biosensor based field screening method might cost £20 per test and even on such low sample numbers, if 20% were found to be negative following field screening, the saving would amount to £5400, ie. the total cost of lab testing minus (the cost of screening all samples + the cost of lab analysis of the positive samples). Given that the EA will invariably analyse far more samples for dioxins than are tested presently by the NRA, eg. soil samples, marine sediments, landfill leachates, fresh water samples, suspect wastes etc., the cost benefits could be very considerable indeed.

Similar arguments could be applied to PCB analysis. Indeed, the use of field immunoassays (rather than biosensors) for the analysis of PCBs in soil has already been shown to yield significant cost benefits (see Bogue 1995).

6.5 Standards

As biosensors are not yet generally available for water quality or broader environmental monitoring applications, the lack of standards is no surprise. The individual responsible for analytical methods at the BSI (Roger Wellings) is aware of no present or planned standardising activities relating to biosensors, in the UK or overseas. There are, however, sub-committees on microbiology (BSI EH/3-4) and biological methods for water quality monitoring (BSI EH/3-5) but neither are concerned with biosensors.

In contrast to the US situation, where the EPA produces standards for environmental monitoring instrumentation and analytical techniques, and accredits the former, there is only one body in Europe producing such standards: the TA Luft in Germany. In the UK and elsewhere in Europe, there is a growing feeling by both the users and instrument manufacturers that

standards and accreditation would be beneficial. Indeed, an earlier survey (GAMBICA/NEDC 1992) revealed that many UK users purchased US EPA or TA Luft approved equipment in favour of non-approved British instruments. Further, the report maintained that, "Certification would put UK firms on a level footing with overseas companies".

However, it is widely recognised by users and manufacturers alike that standards should not inhibit technological developments. The prevailing view in the UK is that any future standards or specifications relating to water quality monitoring instrumentation should address issues such as range, overall measurement uncertainty, and practical considerations such as battery life, data storage capacity and environmental performance (temperature, moisture resistance etc.). A further consideration, being raised in the UK by the users, is cost of ownership. The underlying sensor technology is not an issue.

The whole area of standards for environmental monitoring instrumentation is in the melting pot: GAMBICA is involved with various deliberations in the UK and WRc is investigating certain broader European issues. Whatever the eventual outcome, it is unlikely that biosensors will be implicated directly; they are, after all, merely one of the many classes of sensors that can be used in water quality monitoring instruments.

It is the author's view that standards are not a significant element in any biosensor developments with which the NRA/Agency might become involved. It is unlikely that any standards relating specifically to biosensors will be produced during the foreseeable future.

7. STRATEGIES FOR THE FUTURE

7.1 Preamble

It should now be evident that biosensors could contribute significantly to several aspects of the NRA/Agency's monitoring activities. The most pertinent issue to be resolved is whether the NRA/Agency should act proactively or simply await the availability of commercial products.

The former course of action is recommended, because:

- 1) products need to be tailored to satisfy specific tasks. Without an NRA/Agency input, it is unlikely that products would meet exactly many (or any) of its specific requirements;
- 2) the involvement of the NRA/Agency as a major end-user could catalyze developments which might otherwise be very slow indeed, particularly those of a highly user-specific nature;
- 3) inactivity will result ultimately in many of the regulated companies rather than the regulators having access to the most advanced biosensing technology.

7.2 Strategies

7.2.1 Overview

The proposed strategy aims to ensure that the NRA/Agency has access to biosensor-based products that meet its specific requirements and has a continuing involvement with the technology.

It comprises four elements, as follows.

- 1) Evaluating all potentially useful biosensor products as they become available commercially;
- 2) Supporting collaborative R&D projects, principally providing the user input and focus;
- 3) Evaluating laboratory prototypes and providing research groups with real user feedback;
- 4) Integrating these functions into a unified activity.

Each of these are considered in the following sub-sections.

7.2.2 Evaluating available products

A small number of products already exist (see Appendix B) that could, in principle, find uses within the NRA. They should be evaluated. The NRA/Agency should maintain an awareness of all potentially relevant biosensor products as they become available in the future and evaluate them accordingly.

7.2.3 Collaborative R&D

Whilst, say, five years ago it might have been useful to collaborate with organisations from outside of the environmental sector, perhaps healthcare or food, to establish fundamental, generic biosensing principles or technologies, much of this has now been elucidated and the need now is to stimulate the availability of useable products. Biosensor technology has now passed the stage where "dabbling" is warranted, thus, collaborations should involve sensor manufacturers with commercialisation in mind.

However, it is vital for the NRA/Agency to have clearly defined its needs, both technically and in terms of likely levels of usage, before such projects are entered into. There must be real and attainable goals, ideally useable products.

Collaboration with other European environmental protection agencies or similar bodies is highly desirable as, in addition to reducing the (presently rife) duplication of effort from one country to another, the larger potential markets are far more likely to interest manufacturers than are individual national markets. This point was noted specifically by Bookham Technology with regard to the economic viability of developing disposable field biosensors for pesticides.

Several types of collaboration warrant consideration:

- * UK LINK schemes (DTI supported), which generally involve an end-user, a university and a manufacturer;
- * EC-supported projects, such as those forming part of the Climate and Environment programme (see 5.3.2, above). These involve academic and other partners from three or more member countries;
- * Directly funded collaborations, eg. between the NRA/Agency and one or more universities, and perhaps a manufacturer;
- * Independently arranged international projects, for instance, between an overseas environmental agency, one or more universities and a sensor manufacturer. Costs would be shared between all parties.

Despite the scarcity of available environmental biosensors, several UK companies have expressed a willingness to become, or are already, involved in biosensor manufacture. Thus, identifying a partner to undertake manufacture should be relatively straightforward. Some such companies include:

- Environmental Sensors Ltd. (Cambridge University spin-off)
- Cranfield Diagnostics Ltd. (Cranfield University spin-off)
- Gwent Electronic Materials Ltd. (experts in thick-film materials and technology, fabricating prototype biosensors for UWE)
- Ecosensors Ltd. (developing thick-film biosensors for BOD etc.)

- Bookham Technology Ltd. (developing optical biosensors with Cranfield)
- GEC Marconi (involved with the pan-European BIOPTICAS project)

Other potential partners include some of the larger, diversified companies with broad sensing interests, such as the Bowthorpe group, GEC-Marconi and Siemens-Plessey.

7.2.4 Evaluating prototypes

Evaluating prototypes as they emerge from university research is seen as a vital part of the proposed strategy. Many research groups reach the point where they have demonstrated a biosensor's ability to respond to some analyte but thereafter, progress usually stalls, as the next stages in the road to market (evaluation, modifications, pre-production devices) fail to materialise.

The NRA/Agency could catalyse this process by testing prototypes under real conditions and providing the feedback necessary to take a device to the pre-production stage. Then, a manufacturer could become involved. Universities invariably welcome such inputs, and the proposed testing of prototype biosensors for organophosphorous pesticides from the University of the West of England should be seen as the start of an on-going process.

It is clearly vital to maintain an awareness of biosensor developments that warrant this approach. The research details in this report alone are sufficient to pursue further and immediately this element of the strategy. In the longer term, the many conferences and publications on the topic will provide the necessary information.

7.2.5 Integrating and overseeing

The proposed strategy is unlikely to succeed without the various elements being incorporated into a unified function. This must take into account the monitoring interests of all of the elements of the forthcoming EA.

At the present time, knowledge of, and involvement with, biosensors is spread between several individuals within the NRA and with the broader operational scope of the EA, there is every likelihood of further fragmentation.

7.3 Costs and timescales

It is appropriate to consider finally the likely costs and timescales involved in developing biosensors of the types discussed in this report. In this context discussions were held with the Universities of Cambridge and Cranfield, who have seen the fruits of their research taken to market, and UWE, who so far has not.

7.3.1 Costs

Assuming that the task is to take a working lab prototype to a stage where it could be manufactured readily by an instrument company, figures in the £100-300K were proposed. Costs vary, of course, according to the nature of the sensor, but this estimate ties in with the figure of £200K quoted by Turner (Cranfield) at a recent meeting at the WSA (April 1995) for the thick-film, enzyme-based phenol sensor. Cowell (UWE) suggests a figure of, approximately £100-150K to take his group's prototype thick-film biosensors for OP pesticides to the pre-production stage; a figure that includes the development of the associated electronic instrumentation. Lowe (Cambridge) maintains that developing a biosensor from scratch, for instance, a dioxin sensor to meet a specific NRA requirement, might cost £500K or more.

In the main, these figures are less than those publicised for earlier, successful commercial biosensor developments and reflect two facts: much of the underlying science and technology is now reasonably well established; and, the lower figures are based on the commercialisation of working prototypes. Even when the "from scratch" figure is considered, a collaborative project involving, for instance, the NRA/Agency, an overseas environmental body and a manufacturer, would reduce the individual

financial burden to a not unreasonable level of, say, £100-200K over two years.

7.3.2 Timescales

Timescales are difficult to quantify in general but in the case of either the Cranfield phenol sensor or the UWE pesticide sensor, a period of one to two years is seen as realistic, given adequate funding. This timescale is seen as being generally representative for a device of limited complexity, where a working prototype has been demonstrated and where the fabrication technology does not require significant development. A suitably funded project, starting from scratch, and reaching the pre-production stage, might realistically be expected to take three years, given that there was no fundamentally new science to establish.

The aspect of fabrication technology is important, as biosensors based on, for example, integrated optics (eg. BIOPTICAS); necessitate the development of new and largely unexplored production techniques which would invariably incur significant further development time (and costs).

Should the NRA/Agency decide to accept the recommendations of this study and act on them in the near future, it is quite feasible for it to be using a variety of biosensors on a regular basis well before the close of this century.

8. CONCLUSIONS AND RECOMMENDATIONS

1. Biosensors are a class of molecular sensors that employ biological agents such as antibodies, living cells or enzymes etc. These are maintained in contact with a transducer which generates an output signal which is functionally-related to the concentration of the analyte. They can employ a diversity of biochemical reactions and transduction principles and be based on many solid-state and optical technologies. They have enjoyed a considerable degree of success in the healthcare industries and are now starting to exert an impact on environmental monitoring. However, few products are yet available for this use and most are concerned with the rapid determination of BOD in the laboratory. Many biosensor-based products are under commercial development by both large and small companies and are aimed at the determination of a range of environmental analytes such as pesticides, BOD, toxicity, micro-organisms, metals and toxic gases.

2. Environmental biosensor research is underway at numerous academic institutions throughout the world and addresses many scientific, technological and practical issues and involves a diversity of sensing principles and technologies. Many of the target analytes are of potential interest to the NRA/EA and many developments are at an advanced stage. Several collaborative environmental biosensor projects are being funded by the EU and involve commercial and academic partners from member states. The UK is reasonably well represented amongst these and the NRA is involved in one such project. A fresh round of projects commences each year, jointly funded to the tune of approximately ECU 2 million.

3. The US EPA has a large and active biosensor R&D programme, mostly involving collaborations with American universities. It is investigating a wide range of principles and technologies for the determination of many environmental analytes. To date the NRA's involvement with biosensors has been modest, comprising some work on live cell toxicity sensors and the

aforementioned EU project. However, there is minimal evidence of support for biosensor research by other European environmental protection agencies.

4. Biosensor R&D is weak within most other, non-medical industries in the UK, although some work is being supported by the food industry and the NERC has an interest. The major supporter of environmental biosensor R&D in the UK is undoubtedly the water supply sector and Yorkshire Water, in particular, has a very strong programme. Anglian Water is a shareholder in a Cambridge University spin-off company commercialising environmental biosensors and several of the other water plcs have an involvement with the technology. In the UK, the Netherlands, France and Germany, and perhaps elsewhere in Europe, the companies regulated by the environmental protection agencies are more actively involved with biosensor developments than are their regulators, and are making most of the running.

5. Biosensors are seen as offering strong prospects to the NRA, the WRAs and the forthcoming EA, and their deployment is expected to yield a number of significant operational and economic benefits. They could be used to undertake a wide variety of environmental monitoring tasks, including laboratory screening, field water quality monitoring, fixed site and on-line monitoring, and solid and liquid waste analysis. Longer term prospects may exist for air quality monitoring. The analytes involved are likely to include phenols, pesticides, BOD, metals, PCBs, micro-organisms, toxicity, silage liquor and toxic gases. Standards are not perceived as having a bearing on any biosensors with which the NRA/EA might become involved.

6. It is argued that, for the NRA/EA to become most meaningfully involved with biosensors, it must act proactively. A strategy is proposed that comprises the evaluation of existing biosensor products, collaborative R&D, evaluating laboratory prototypes and overall strategy management. This approach will: speed up commercial developments; make available products that meet exactly the NRA/EA's needs; and, ensure that it has access to similar sensing technology to that of the companies it is regulating.

7. Collaborations involving continental European partners are recommended although LINK schemes and other options warrant consideration. A number of environmental and other organisations in the UK and overseas have expressed an interest in collaborating with the NRA/Agency. Many UK and European academic institutions possess exploitable biosensor technology and several UK companies are well placed to act as manufacturers. Involving manufacturers with a view to commercialisation is seen as vital, as collaborations must aim to yield useable products. Taking a laboratory prototype to production is likely to take from one to three years, with a cost in the region of £100-300K.

8. It is recommended that the proposed strategy be adopted. Implementation of the strategy will not only benefit the NRA/EA; it will also help UK sensor manufacturers to profit from the UK's undisputed expertise in biosensor technology.

APPENDIX A - Affiliations of contributing individuals

Birkbeck College, London
Bookham Technology Ltd.
British Gas, Loughborough Research Station
British Standards Institute
Dr Bruno Lange (UK) Ltd.
CEC, DG-XII, Belgium
Cranfield University
Danish Environmental Protection Agency
European Environmental Protection Agency (Denmark)
Gesellschaft fur Biotechnologische Forschung (GBF), Germany
GEC-Marconi, Hirst Research Centre
HMIP (Monitoring Branch, Business Strategy and Information Division)
Institute of Food Research (IFR), Norwich
International Centre of Water Studies, Netherlands
Irish Environmental Protection Agency
J T Baker Ltd. (UK Ohmicron agent)
Leatherhead Food Research Association
MAFF, Chief Scientist's Group
NRA
NERC
Plymouth Marine Laboratory
RIVM, Netherlands
Sartorius Ltd. (UK Biosensori agent)
STOWA (Foundation for Applied Water Research), Netherlands
SWIG
University of Cambridge
University of the West of England

APPENDIX B - Details of commercially available environmental biosensors

MIDAS PRO

Manufacturer	Biosensori SpA, Italy
Analyte	Microbial numbers (in water)
Principle	Mediated amperometry
Response time	10 minutes
Resolution	$10^5/10^6$ cells/ml
Range	No upper limit
Price	£4.5K (hardware), consumables c. £3/test
Applications	Monitoring bacteria in sewage works, drinking water etc.
Comments	Technology developed at Cranfield University
Available in UK	Sartorius Ltd. Tel. 01372-745811

Biosensor BOD

Manufacturer	Bruno Lange GmbH, Germany
Analyte	BOD
Principle	Measuring oxygen consumption of immobilised micro-organisms with oxygen electrode
Response time	2 minutes
Resolution	Not quoted
Reproducibility	c. 5%
Range	6 - 600 mg/l BOD
Price	£9,695
Replacement head	£114.50 (biosensor membrane)
Applications	Rapid BOD determinations in the laboratory
Comments	Technology developed originally at a German university
Available in UK	Dr Bruno Lange (UK) Ltd. Tel. 01276-677233

BSB Module

Manufacturer Prufgerate-Werk Medingen GmbH, Germany
Analyte BOD
Principle Oxygen consumption of micro-organisms measured with an oxygen sensing membrane
Response time Less than 3 minutes
Resolution Not quoted
Range 2 - 22 mg/l BOD
Applications Rapid BOD determinations in the laboratory
Available in UK No known UK outlet. German tel. 0049-351-64830

BOD 2000, BOD 2200

Manufacturer Central Kagaku Corp, Japan
Analyte BOD
Principle Measuring oxygen consumption of immobilised micro-organisms with oxygen electrode
Response time No data available
Resolution, range No data available
Applications 2000 is for lab use, 2200 is for fixed field monitoring
Available in UK No known UK outlet. Japanese tel. 0081-3-3812-9186

Pesticide Analyzer

Manufacturer SERES SA, France
Analyte Pesticides (triazines)
Principle On-line immunoassay, colourimetric detection
Response time c. 45 minutes
Resolution Not quoted. Assumed to be less than 0.05 ppb
Range 0.05 - 1.00 ppb
Price To be established
Applications On-line analysis of triazines in water
Available in UK SERES (UK) Ltd. Tel. 01344-762211

MEG Biosensor

Manufacturer British Gas, Loughborough, UK
Analyte Monoethylene glycol (MEG)
Principle Enzyme reaction, oxidation of liberated NADH detected
amperometrically with coated platinum electrode
Resolution 2 mg/m^3 (MEG in air)
Range Up to hundreds of mg/m^3
Applications Measuring MEG levels during gas wetting. Possible uses in
monitoring de-icing fluid run-offs at airports
Comments 14 instruments manufactured and used. No further
production planned. Developed in collaboration with UK
universities
Available in UK N/A. Contact Dr R Newell (British Gas), tel. 01509-282271

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