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Review

Biosensors and Ecotoxicology

Sensors and biosensors as well as ecotoxicological tools like bio-analytical systems, bioassays and biomarkers provide us with detection systems for signaling a potential damage in the environment (environmental signaling). These responses of early recognition will prevent the eventual damage in the environmental matrices. Once an ecosystem damage has occurred, the remedial action processes for recovery could be expensive and pose certain logistical problems. Ideally, "early warning signals" in ecosystems using sensing systems (biosensors) and biochemical responses (biomarkers) as well as the classical effect-related bioassays would not only tell us the initial levels of damage, but these signals will also provide us with answers for the development of control strategies and precautionary measures. In order to understand the complexity of the structure of populations and processes behind environmental health, our efforts have to be directed to promote rapid and cost-effective new emerging parameters, such as effect-related parameters like biochemical responses (biomarkers) in the field of immunotoxicity and endocrine disruption. Environmental effects, e.g. genotoxicity, were detected in organisms from various "hot spots" and UV-B exposed fish embryos. One problem is always to find the relevant interpretation and risk assessment tools for the environment in the context of the reference areas.

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1 Introduction

For ecotoxicological tools and risk assessment, new recommendations are described in the Technical Guidance Document of the European Commission (EU), Edition 2, in the new EU Chemicals Legislation REACH and in the status report for toxicological methods of the European Center for the Validation of Alternative Methods. In the EU water framework directive [1], a general requirement for ecological protection, and a general minimum chemical standard was introduced to cover all surface waters. For the description of a "good chemical status", *Effects-Monitoring Tools* such as biosensors [2–5] are needed for the description of the status of river basin systems [6, 7]. In some cases, biomarkers and biosensors are helpful to promote an environmentally sensitive and sustainable use in studies with coastal zone samples [8] for coastal zone management.

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Biomarkers are already being applied to the water matrix and to organisms. This refers also to emerging biomarkers and bioanalytical systems for detecting endocrine and immunotoxic effects. The usefulness for new promising effect parameters such as immunotoxic (phagocytic) ones in the context of biotoxins is demonstrated by mussels exposed to sediments in coastal areas and exposure of mussels under controlled conditions to the water matrix. Results are available for immunotoxic effects in mussels in the context of the endocrine load of the sediments demonstrated in several investigations in fresh water and coastal areas [9, 10–13]. The immunotoxic response was quantified by the phagocytic activity (Phagocytic Index) of the blue mussel hemocytes [14, 15]. It appears that both immunosuppressive and immunostimulative effects are likely to occur at specific sites and that responses will be influenced by the type and intensity of contaminants present. The function of the immune system in bivalves can be adversely affected by long-term exposure to environmental contaminants. Investigating alterations in immunity can therefore yield relevant information about the relationship between the exposure to environmental contaminants and the susceptibility to infectious diseases. Detecting and quantifying of infectious materials and biotoxins in exposed mussels is a directing field of biosensors for the future.

2 Biochemical Responses and Biosensors for Monitoring the Environment

The use of biomarkers (biochemical responses) in multi-arrays for environmental monitoring is complementary to chemical analysis since biomarkers can detect the presence of toxic compounds that require further instrumental analysis or "bio-response-linked instrumental analysis". Biosensors are by definition [16] analytical devices incorporating a biological component like microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids and a physicochemical transducer system. An enzyme-linked recombinant receptor assay, for example, is a biosensor according to this definition: the biological component is the enzyme and the transducer is the optical component. Biosensors together with effect-related parameters or biochemical responses for monitoring the environment are complex but they will give a clear picture of the health status of the investigated system. Enzyme biosensors are sensors based on enzymatic bio-recognition elements. Therefore the definition by the International Union of Pure and Applied Chemistry (IUPAC) can be widened: "Biosensors are analytical devices incorporating a biologically derived and/or bio-mimicking material (i.e. cell receptors, enzymes, etc.), associated with or integrated within a physicochemical transducer or a transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric, or magnetic [16]. Biosensors are distinct from bioassays in that the transducer is not an integral part of the analytical system.

Biosensor and biochemical responses for the assessment of environmental health are listed in Tab. 1. It is rather difficult to transfer the monitored biochemical responses or the sensor endpoint responses into an operational effect-related standard (EQS and QN = Environmental Quality Standards and Quality Norm) for environmental monitoring. Sensing systems based on the induction and inhibition of a functional system relating to function, interference and effect-related endpoints are listed in Tab. 1.

The effect-related endpoints quantify the health status of the investigated environment. The so-called "*Ecosystem Health*" is

Table 1. Environmental monitoring of effect-related biochemical responses (biomarkers) and potential sensing systems with the biological recognition element of the assay/sensor and their endpoints ("effects at the level of" neurotoxicity, etc.): Modified according to [17].

Biological recognition element of the sensor	Example	Detection of compounds	Endpoints
Proteins Enzymes	Acetylcholine esterase	Organophosphorus and carbamic compounds	Neurotoxicity
	Protein phosphatase 1 and 2A	Microcystins	Hepatotoxicity
Ion channels	Na^+ channel (voltage-gated channel)	Saxitoxin, tetrodotoxin, procaine	Neurotoxicity
Transport protein	SHBG, CBG, TBG	Endocrine disruptors	Growth, reproduction
Receptors	Estrogen receptor	Endocrine disruptors (e.g. o,p-DDT, nonylphenol)	Growth, reproduction
	Nicotinic acetylcholine (ACh) receptor (ligand-gated channel)	Anatoxins	Neurotoxicity
Electron carriers	QB protein	Photosynthesis II herbicides (e.g. *s*-triazines, phenylureas), phytotoxins	Photosynthesis
Nucleic acids DNA	DNA double strands	PAHs, pesticides, PCBs, EDCs, intercalating polycyclic aromates (ethidium, acridine, caffein); DNA adducts (metabolites of chloracetamide herbicides)	Genotoxicity
Cytoskeleton	Tubulin	Colchicin, taxol; anti-tubulin herbicides (e.g. trifluralin, oryzalin)	Cytotoxicity
Ribosomes	rRNA (ricin)	Ribotoxins (ricin, abrin, Shga toxin)	Cytotoxicity

synonymous with "*Environmental Integrity*", from which it follows that the scope of Ecosystem Health (EH) research encompasses all the ecotoxicological tools and approaches which are efficacious in increasing the cognitive, curative, and preventive knowledge as the goal to the preservation of environmental integrity. *Ecosystem Health Research* thus directs its attention to the prediction of reversible and irreversible insults which human or other activities could potentially inflict onto the environment. For the assessment of ecosystem health there are very promising bioassays and biomarker approaches (biochemical responses) which are centered on quantifying biochemical effects in organisms and populations. One important tool for the acceptance of bioassays and biomarkers in science, technology and governmental legislature are the so-called inter-laboratory comparison and validation studies. In many intercalibration investigations, laboratory studies have demonstrated a strong causal link between exposure of fish to PAHs and co-planar PCBs and the expression of cytochrome P_{450}1A1 and its associated EROD activity (7-ethoxyresorufin-O-deethylase). The induction of EROD activity in fish liver has particularly been used as a biomarker for the effects of these organic contaminants.

Beside the EROD induction as a classical biomarker for biotransformation, another investigated biochemical response is the cholinesterase inhibition (ChE). Organophosphorus pesticides and carbamates cause the inhibition of cholinesterase and stands for neurotoxicity. For the quantification of neurotoxicity there are two well-known cholinesterases (acetyl cholinesterase and butryl cholinesterase). The methology is standardized according to DIN (German Institute for Norming: DIN 38415-T1 1995). Because of the validated method "Cholinesterase Inhibition", several biosensor systems were developed and commercialized.

There is an application of validated biomarkers available with endpoints including new emerging biomarkers for the detection of endocrine effects and immunotoxic (phagocytic) parameters in addition to genotoxic ones. There is a high potential of biochemical responses and the development of fast and reliable biochemical tools (biosensors) for on-site screening in environmental and human health analysis.

3 The River System Example of Berlin Classified by a Biosensor System

The following development should serve as an example for the application of a biosensor system detecting vitellogenin in the organisms by a competitive immunoassay and by the ELRA assay [18] in the sediments.

Endocrine effects in the sediments of the River Havel, Spree, Dahme and Teltowkanal are calculated and expressed as 17β-Estradiol equivalents [μg/kg] in sediment dry weight and for elutriates of sediments in [μg/L]. The lower River Havel shows a median concentration of 8.5 μg/kg of 17β-Estradiol equivalents, and the upper River Havel 6.7 μg/kg of 17β-Estradiol equivalents; 34.2 μg/kg of 17β-Estradiol equivalents were found in the River Spree, 45.7 μg/kg of 17β-Estradiol equivalents in the River Dahme and in the Teltowkanal 14.3 μg/kg of 17β-estradiol equivalents. The results in Fig. 1 clearly demon-

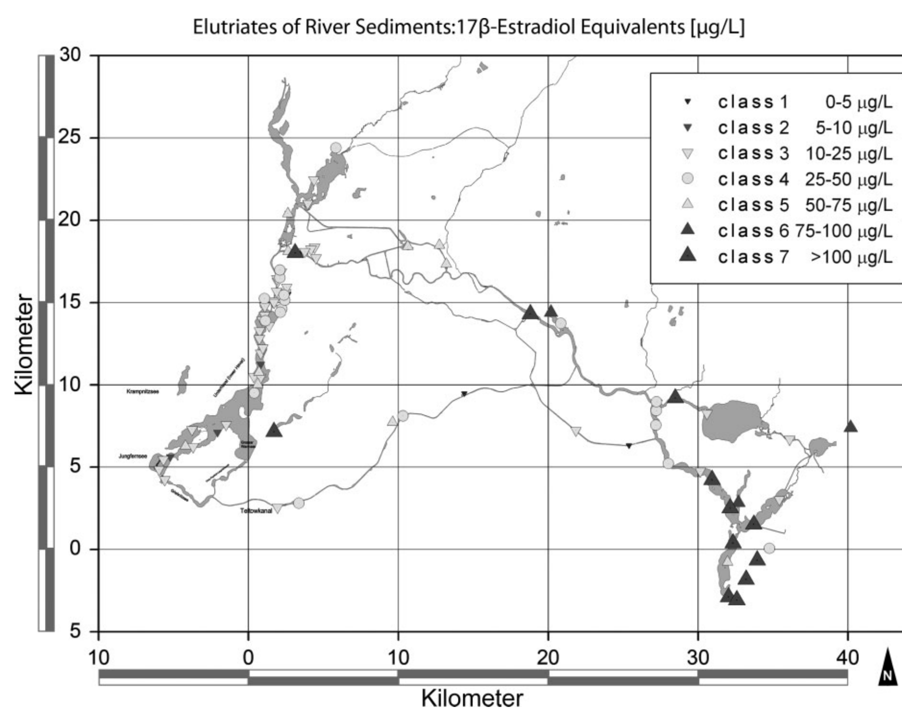

Figure 1. 17β-Estradiol equivalents [μg/L] for elutriates of sediments in the Berliner River system using the ELRA system. Sediments were sampled at the top 0–5 mm layer of the water way [13, 18].

strate that concentration levels up to 175 μg/L of 17β-Estradiol equivalents in elutriates of sediments are coming from sites with an extreme suspension rate. In Figs. 2 and 3, the concentration levels of 17β-Estradiol equivalents were divided into in seven classes. Endocrine effects measurements were carried out by the ELRA-test (ELRA = *Enzyme Linked Recombinant Assay*).

Analyses performed by ELRA (*Enzyme Linked Recombinant Receptor Assay* [18–21] and the hER and hAR assay by McDonalds will be soon part of an intercalibration study for endocrine substances according to the German Institute for Norming (DIN). Sediment loading by xenoestrogen changes over the year is also due to extreme algal growth on the one side, and a substantial amount of phytohormones, on the other. Apart from estrogenic responses there is only a poor evidence of androgens in River Havel sediments.

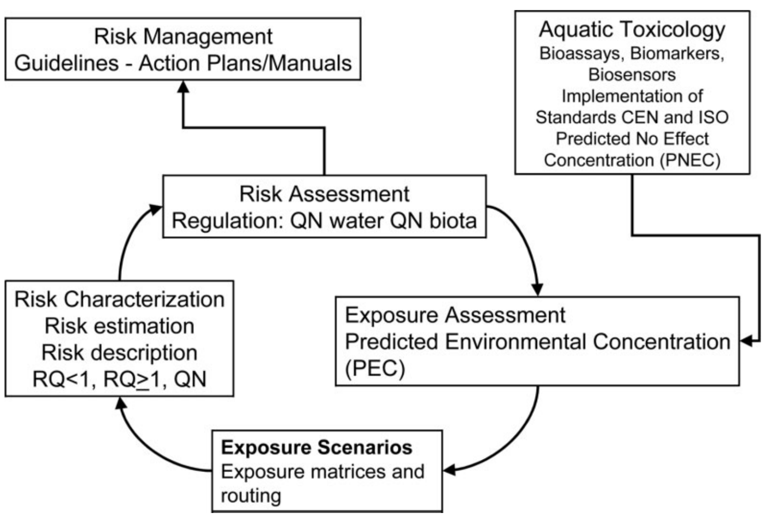

Figure 2. Risk Assessment and Quality Norms (QN) by bioassays and biosensors (exposure and effect calculation) [22, 23].

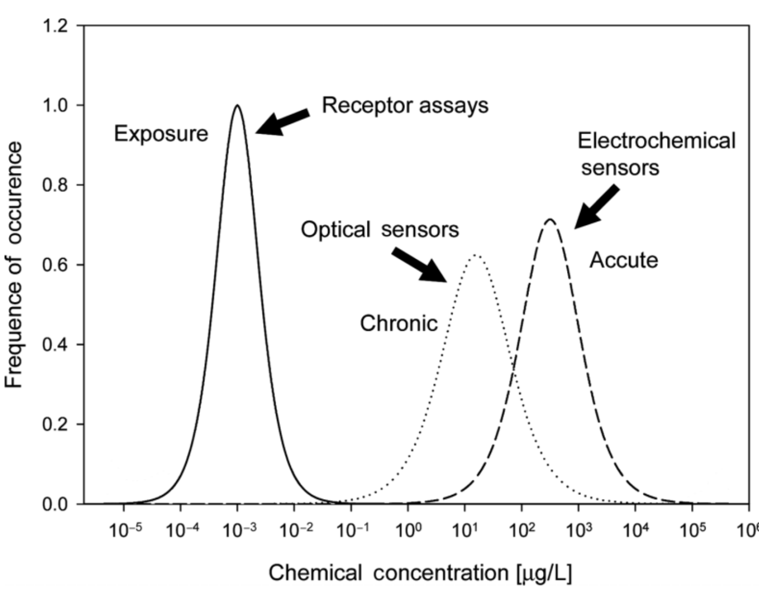

Figure 3. Exposure concentrations and the effect-related responses of the exposure concentrations by bioassays: chronic bioassays (NOEC), acute bioassays and the sensitivity of the state-of-the-art biosensors, such as receptor assays, optical sensors and electrochemical sensors for the detection of inorganic (metals) and organic compounds.

4 Discussion

A relevant question meriting discussion is the following: Can biosensors give additional information? For on-line measurements, there are some doubts that "state-of-the-art" biosensors will give additional information and will replace chemical analytics or even bioassays. The case study emphasized the need to develop biosensors which do not only measure "conventional" contaminants but also new emerging parameters like endocrine and immunotoxic effects which are needed for compliance with the drinking water directive as well as the water frame work directive (WFD) and which are currently used in monitoring programs and regulative bodies [22]. The classification of water ways in the context of endocrine effects in Fig. 1 expressed as 17β-estradiol equivalents contributes to the evaluation by receptor-assay sensors for the environmental risk assessment (ERA), risk communication and risk management [22, 23].

To exploit the principal advantage of biosensors, standardization and harmonization is needed for governmental decision making in collaboration with industry and governmental authorities. Very promising is the additional information available especially in the field of bio-effect related sensors like whole-cell sensors and receptor sensors. Progress is being made in the development of receptor-assay sensors [13, 18, 21]. The format of the receptor assays has changed meanwhile to nanotechnology levels, already well established in water and food analysis. The "Quality Norms (QN)" developed after the WFD in correspondence to the "good chemical status" has to be enlarged for endocrine disrupting compounds, as it was shown in this study for estrogens (i.e. 17β-Estradiol) and xenoestrogens (i.e. Nonylphenol and Bisphenol A). Other classes of concern and their potentially hazardous environmental effects should also be investigated by bioanalytical systems like biosensors.

The case study on a receptor-assay sensor and sediments in Berlin water ways showed clearly that biosensors are complementary to chemical methods since they can signal the presence of toxic (i.e. xenoestrogens) compounds that require further effect-related chemical analytical studies. In addition, this will allow to find the correlation of toxicity biosensor data with individual chemical compounds. Because of this cross activity, biosensors can detect chemical compounds which were not target analytes but are detectable by the cross-active biosensors or by the bio-effect-related sensor but not by the chemical instrumental system [2].

In order to protect aquatic organisms and fish populations in the environment, the ecotoxicological data required are the so-called NOEC data (*"No Observed Effect Concentration"*). A compensation, uncertainly or safety factor has to be introduced to take into account the uncertainty associated with extrapolating results to the real environment.

As a general principle, the lowest test result for the most sensitive species has to be used as the starting point for the risk assessment and for the derivation of the water and biota quality norms (QN). The toxicity data used for the risk assessment have to be examined critically with respect to validity and relevance.

The environmental risk assessment in urban systems has two major elements: characterization of effects and of exposure. The Strategic Dimension of Environmental Resources and Quality encompasses the fields of Integrated Water Resource Management, Environmental Impact Assessment and Ecological Risk Analysis.

In a close integration of ecosystem related aspects during the planning process of urban projects and in the urban design criteria, defining the sustainable quality criteria as the dominant research questions are mainly concerned with the possibilities of reducing water consumption without decreasing the comfort of living combined with an integration of *Environmental Impact Assessment* (EIA) and an *Ecological Risk Analysis*.

The Environmental Quality Standards (EQS) and their quality norms (QN) require a testing whether or not sufficient measures have been taken to protect the individual body of water and especially its valuable and exploitable resources from "dangerous substances". In order to secure the functioning and stability of the ecosystem of i.e. a body of water (self-purification capacity) or to actually re-establish these qualities, concrete ecological knowledge is necessary concerning the interactivity of functions and structures of the living systems under these extreme conditions, as well as nutrient cycles and energy fluxes in their temporal succession.

The environmental quality norms serve as management tools (see Fig. 2) for Environmental Quality, i.e., for protecting the waterways as a valuable natural source supporting drinking water, human health, outdoor recreation, and in general the ecosystem health in Urban Areas especially Mega-cities. The bio-monitoring tools are used to characterize exposure data and potential effects; their use has to be characterized and possibly tailored for different types of environmental media.

The resource management contributes to the evaluation and the interaction of ecosystems and to the urbanization as well as to the quantification of risk. The environmental risk assessment in urban systems has two major elements: characterization of effects and of exposure. The generated data and investigations concerning monitoring in support of Environmental Risk Assessment (ERA) have to be adequate for the characterization of exposure and effects indicating the sustainable development for ecosystem and human health protection in Megacities. The objectives have to be achieved by *Effect-Related Monitoring* and "*Effect Indicators*" for the best quality of life/environment monitored in a benchmarking process.

In considering the impact of either natural stress or manmade stress, one always encounters detoxification, disease defense, regulation, and adaptation processes. This situation makes the assessment approach by biomarkers rather complicated. The biomarkers do have a significant ecological assessment potential not only in the symptom analysis including both functional (behavior, activity and metabolism) and structural changes in organisms (cellular, tissue and organs). For landscape planning and environmental management it is necessary to obtain significant data from biochemical responses for relevant and sustainable actions. As an example of transfer of new substances, i.e. from sediments to groundwater, the case study of the River Havel case study (see Fig. 1) in Germany demonstrates a close relation between environmental monitoring and the ecosystem health aspects.

Biosensors along with the effect-related parameters or biochemical responses for environmental monitoring are very complex but they will give a clear picture of the health status of the investigated system.

5 Summary

Biosensors are early recognition systems that indicate the presence of unknown compounds which are responsible for the signals they detect. Progress has been made with the development of receptor assays and their sensitivity (see Fig. 3) close to the exposure concentrations in the environment.

Biosensors offer many advantages for environmental monitoring but they have to meet the standards of the well standardized and harmonized eco-toxicological assays under CEN and ISO.

Biosensors are complementary to the chemical methods since they can alert the presence of toxic compounds that require further effect-related chemical analytical studies. In addition, this will allow to find the correlation between toxicity biosensor data with individual chemical compounds (REACH).

For diagnosis concerning human health, new technologies like proteomics, metabolomics and genomics are presently in developmental stages. These tools will eventually help to validate effects in humans and in the environment and this could be one relevant direction for the future.

Many biosensor techniques have been developed to meet the demands of the lucrative biomedical markets (97 % in the global marked place) and await an adaptation for environmental applications, while the demands of continuous on-line monitoring are still facing problems that will require unique solutions.

References

[1] *Directive 2000/60/EC of the European Parliament and of the Council Establishing a Framework for the Community Action in the Field of Water Policy.* (http://eur-lex.europa.eu/LexUriServ/site/en/oj/2000/l_327/l_32720001222en00010072.pdf)

[2] S. Alcock, D. Barcelo, P.-D. Hansen, Monitoring freshwater sediments, *Biosensors Bioelectronics* **2003**, *18*, 1077–1083.

[3] P.-D. Hansen, J. Blasco, A. De Valls, V. Poulsen, M. van den Heuvel-Greve, Biological analysis (Bioassays, biomarkers, biosensors), in *Sustainable Management of Sediment Resources*, Vol. 2: *Sediment Quality and Impact Assessment of Pollutant*s (Eds: D. Barceló and M. Petrovic), Elsevier Publishers, Amsterdam, London, New York **2007**, 131–161.

[4] S. Rodriguez-Mozaz, M.J. Lopez de Alda, M.-P. Marco, D. Barceló, Biosensors for environmental monitoring a global perspective, *Talanta* **2005**, *65*, 291–297.

[5] A. Lange, M. Maras, W.M. De Coen, Bioassays and biosensors: Capturing in a nutshell, in *Ecotoxicological Testing of Marine and Freshwater Ecosystems: Emerging Techniques, Trends and Strategies* (Eds: P.J. den Besten, M. Munawar), Taylor and Francis, Boca Raton, London **2005**, 177–190.

[6] P.-D. Hansen, H.-J. Pluta, J. A. Beeken, Biosensors for on-line monitoring of the waterways and for sample taking aimed at relieving disturbances, *GBF Monogr.* **1989**, *13*, 113–116.

[7] P.-D. Hansen, On-Line Monitoring mit Biosensoren am Gewässer zur ereignisgesteuerten Probenahme, *Acta Hydrochim. Hydrobiol.* **1992**, *20 (2)*, 92–95.

[8] P. Baumard, H. Budzinski, P. Garrigues, H. Dizer, P.-D. Hansen, Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: Occurrence, bioavailability and seasonal variation, *Marine Environ. Res.* **1999**, *47*, 17–47.

[9] N.S. Arab, E. Lemaire-Gony, E. Unruh, P.-D. Hansen, B.K. Larsen, O.K. Andersen et al., Preliminary study of responses in mussels (*Mytilus edulis*) exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether, *Aquat. Toxicol.* **2006**, *78 (Suppl. 1)*, 86–92.

[10] C. Blaise, F. Gagne, M. Salazar, S. Salazar, S. Trottier, P.-D. Hansen, Experimentally-induced feminisation of freshwater mussels after long-term exposure to a municipal effluent, *Fresenius Environ. Bull.* **2003**, *12 (8)*, 865–870.

[11] P.-D. Hansen, V. Usedom, New biosensors for environmental analysis, in *Frontiers in Biosensorics II* (Eds: F.W. Scheller, F. Schubert, J. Fedrowitz), Birkhäuser Verlag, Basel, Boston, Berlin **1997**, 109–120.

[12] P.-D. Hansen, H. Dizer, B. Hock, A. Marx, J. Sherry, M. McMaster, Ch. Blaise, Vitellogenin – a biomarker for endocrine disruptors, *Trends Anal. Chem. (TRAC)* **1998**, *17 (7)*, 448–451.

[13] P.-D. Hansen, Biosensors for environmental and human health, in *Advanced Environmental Monitoring* (Eds: Y.J. Kim and U. Platt), Springer Publisher, Heidelberg, New York **2007**, *4 (23)*, 297–311.

[14] P.-D. Hansen, R. Bock, F. Brauer, Investigations of phagocytosis concerning the immunological defense mechanism of *Mytilus edulis* using a sublethal luminescent bacterial assay (*Photobacterium phosphoreum*), *Comp. Biochem. Physiol.* **1991**, *100C (1/2)*, 129–132.

[15] P.-D. Hansen, E. Unruh, TRIPLE LUX-B: Phagocytosis in mussel hemocytes, in *Proceedings of the 9th European Symposium on Life Sciences Research in Space,* 26th Annual International Gravitational Physiology Meeting, Cologne, Germany, 26 June–1 July 2005 (ESA SP-585, August **2005**).

[16] D.R. Thévenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: Recommended definitions and classification (IUPAC Technical Report), *Pure Appl. Chem.* **1999**, *71 (12)*, 2333–2348.

[17] U. Bilitewski, G. Brenner-Weiss, P.-D. Hansen, B. Hock, E. Meulenberg, G. Müller et al., Bioresponse-linked instrumental analysis, TRAC, *Trends Anal. Chem.* **2000**, *19 (7)*, 428–433.

[18] M. Seifert, S. Haindle, B. Hock, In vitro analysis of xenoestrogens by enzyme linked receptor assay (ELRA), *Adv. Exp. Biol.* **1998**, *444*, 113–117.

[19] R. Kase, P.-D. Hansen, B. Fischer, W. Manz, P. Heininger, G. Reifferscheid, Integral assessment of estrogenic potentials of sediment-associated samples, Part 1: The influence of salinity on the in vitro tests ELRA, E-Screen and YES, *Environ. Sci. Pollut. Res.* **2007**, DOI: http://dx.doi.org/10.1065/espr2007.06.429

[20] R. Kase Robert, P.-D. Hansen, B. Fischer, G. Reifferscheid, W. Manz, Integral assessment of estrogenic potentials of sediment-associated samples, Part 2: Integral assessment of estrogen and anti-estrogen receptor binding potential of sediment associated chemicals under different salinity conditions with the salinity adapted enzyme linked receptor assay, *Environ. Sci. Pollut. Res.* **2007** (in press).

[21] P.-D. Hansen, J. Koehler, D. Nowak, 1st and 2nd European Technical Meetings, *Biosensors for Environmental Monitoring*, Berlin **2000**, ISSN 1617–1721, 193 pp.

[22] J. Wharfe, D. Tinsley, M. Crane, Managing complex mixtures of chemicals – a forward look from the regulators' perspective, *Ecotoxicology* **2004**, *13*, 485–492.

[23] P.-D. Hansen, Risk assessment of emerging contaminants in aquatic systems, TRAC, *Trends Anal. Chem.* **2007**, *26 (11)*, 1095–1099.

