

4 Bioassays as a tool for the assessment of the quality of dredged material

The application of bioassays for the assessment of dredged material is advised by the dredged material management guidelines of international conventions (chapter D 4.2). However, on a national level the implementation of bioassays for the purpose of dredged material management is still under development. In the Netherlands a number of bioassays are evaluated and their implementation is scheduled for 2002 (chapter D 2.4.4).

Issues concerning bioassays with regard to dredged material management have been discussed by international experts and stakeholders during two workshops - a science-orientated and a policy-orientated workshop - which were organised as part of this project (Gandrass, J. et. al, 2000; Salomons & Turner, 2000; published as separate volumes).

The intention of the following chapters is to provide somewhat broader information on bioassays to the reader, being less familiar with these issues, thus complementing the conclusions and recommendations given in the workshop reports. The information given focuses on a general classification of bioassays, their fields of application and related requirements. The implementation of bioassays for regulatory purposes, e.g. notification of chemicals or whole effluent assessment, have a direct or indirect impact on surface water quality including sediments. Approaches combining bioassays and chemical analysis are shortly discussed in the context of hazard and risk assessment of sediments/dredged material. Finally future perspectives are addressed.

4.1 Classification of bioassays

Bioassays are one of the main tools in ecotoxicological assessments. Ecotoxicology has the task to examine effects of chemicals or environmental samples on species, biocoenoses and ecosystems (SRU, 1987). Results of ecotoxicological research constitute the main scientific background for setting immission standards for the protection of the environment (Peters, 1999).

Bioassays (ecotoxicity tests, biotests) are defined as methods which use living cells, tissues, organism or communities, defined in type and amount, to assess exposure-related effects of chemicals (Fent, 1998). Bioassays can be classified with regard to their area of application (laboratory or field conditions), the level of biological organisation and its duration of exposure (Forbes & Forbes, 1994; Klein & Debus, 1994; Rudolph & Boje, 1986).

The scope of bioassays with regard to the level of biological organisation is broad, comprising test systems from the molecular to the ecosystem level. In general three different test levels with regard to the biological structure can be distinguished:

- I. Sub-organism level: tests with sub-cellular complexes, cells, tissues and organs
- II. Organism level: tests with whole organisms of one species (mono-species tests) and its population
- III. Ecosystem level: multi-species tests as micro- or mesocosm and field studies

Due to the different levels of biological organisation, experimental approaches and ecotoxicity parameters, so-called 'endpoints', differ (table 4-1).

Table 4-1: Overview of levels of biological organisation, examples for experimental approaches and test parameters ('endpoints') (Peters, 1999, modified after: Rieß et al., 1995; Steinberg et al., 1999; Fent 1998; Smolka & Weidemann, 1995; Pratt, 1990; Nusch, 1996)

Level of biological organisation of the test-system	Experimental approach	Test parameter ('endpoint')
I. Sub-organism level		
sub-cellular complexes	DNA/chromosomal analysis, enzyme tests, membrane/organelle function	DNA aberration, cell/substance metabolism, membrane/organelle metabolism
cells	in vitro (cell cultures)	mutagenicity, cell metabolism, biomarker, cell proliferation rate
tissues/organs	histology, physiology	cancerogenicity, growth inhibition
II. Organism level		
organisms/populations	mono-species tests: - laboratory - field	mortality reproduction growth consumption behaviour immobilisation teratogenicity metabolism bioaccumulation
III. Ecosystem level		
biocoenoses ecosystems	multi-species tests: - microcosm - mesocosm ecosystem field study	<u>ecosystem function:</u> - primary production - biomass production - respiration - energy flow - nutrients flow - community dynamics - adaptation and regeneration after disturbance <u>ecosystem structure:</u> - population density, structure and dynamics - species abundance - biomass - organism size - biodiversity - interaction (predator-prey/competition, parasitism) - trophic structure - biomagnification

From the sub-organism to the ecosystem test level complexity increases. Consequently, the results allow different assessments of the ecotoxicological potential of a substance or environmental sample. Nevertheless, the effects on different levels are closely related.

They can be described as primary effects, which occur on the sub-organism level, secondary effects (organism level) and consequent effects (ecosystem level) (Nusch, 1991). Most common are tests on the organisms level, so-called mono-species tests (Forbes & Forbes, 1994).

Furthermore, bioassays can be distinguished according to the duration of exposure as acute, sub-chronic (prolonged) and chronic. The duration has to be defined in relation to the duration of the life-cycle of the test species. For most of the vertebrates and invertebrates acute toxicity tests take a maximum of 96 hours. Sub-chronic (prolonged) exposures generally cover less than one reproduction cycle, while chronic exposures continue over one or more life-cycles.

4.2 Fields of Application

Bioassays are used in different fields of application and have to fulfil different tasks. In general, five fields of application of bioassays can be distinguished: regulatory purposes, effect screening, biomonitoring and early warning systems, research and teaching, and hazard/risk assessment. The different fields may overlap, e.g. regulatory purposes and hazard/risk assessment. In the following, a short description of the fields of application is given.

Regulatory purposes

Although the structure of environmental laws and regulations internationally varies (e.g. chapter D 2 and 3), one may summarise that the aim and level of environmental protection is defined in national environmental laws by referring to environmental standards. Environmental standards can be divided in standards, which define the level of acceptable emissions (emission standards) and those, which determine the acceptable level of immissions (immission standards, environmental quality standards, quality criteria or quality objectives (chapter D 1). Bioassays are used to define and control the application of both standards, especially immission standards (figure 4-1).

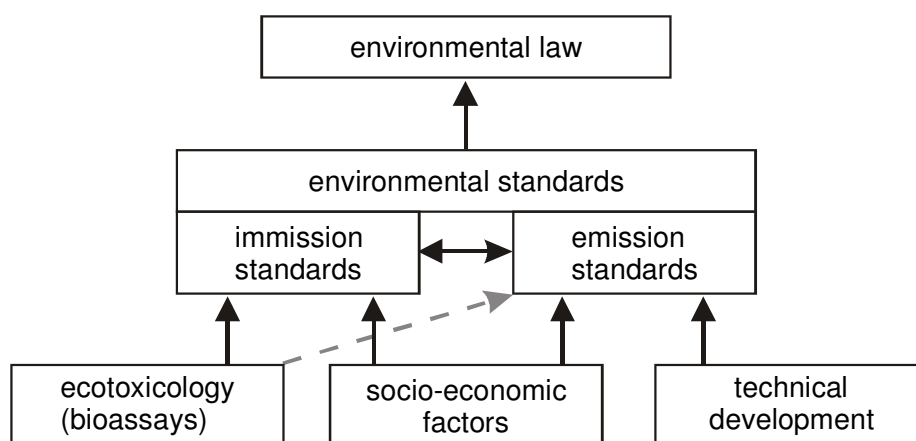


Figure 4-1: Scheme of the role of ecotoxicology (bioassays) in regulations/environmental law (Peters, 1999)

They constitute besides other ecotoxicological studies the main scientific base for immission standards; furthermore socio-economic factors and technical developments are considered (Peters, 1999). They are also used to control emission standards referring e.g. to the

application of best available techniques (BAT) for effluents (German Water Management Act, see 4.4 and D 3).

A more detailed overview of the application of bioassays for regulatory purposes will be given in chapter 4.4. Regulatory purposes may also overlap with other mentioned fields of application, e.g. with hazard/risk assessment.

Effect screening

In ecotoxicological studies, which have to cover large number of samples, bioassays are often applied in order to get a first overview of toxic potentials (effect screening). A rough classification into toxic and non-toxic samples can help to direct the following more detailed investigations in a cost-effective manner. These subsequent investigations usually include again ecotoxicological methods as well as chemical analysis.

Biomonitoring and early warning systems

Biomonitoring has the aim to record and evaluate the status of the environment. Therefore bioindicators are used. Bioindicators are organism, which either respond sensitively when environmental conditions are changing or which accumulate substances (Plachter, 1991; Klein & Debus, 1994). An *early warning system* is a continuous type of biomonitoring using sensitive bioindicators with the aim to detect contaminations early, e.g. due to accidental releases of hazardous substances into the environment.

Research and teaching

The kind of application of bioassays in research and teaching varies, but it can be summarised that the general aim of their application is to gain knowledge about the effect of substances on ecosystems.

Hazard/risk assessment

Another field of application of bioassays is the hazard/risk assessment of a substance or sample. While for hazard assessment the toxic potential of a substance or sample is evaluated, risk assessment should predict the effects of the substance or sample in an ecosystem.

4.3 Requirements and Performance

As shown in the previous subchapter, the tasks which bioassays have to fulfil are different. Consequently, the requirements for bioassays differ as well. The requirements can be described with ten parameters, which are of different importance depending on the field of application. The ten parameters are: ecosystem-relevance, sensitivity, reproducibility, standardisation, practicability, suitability, public perception, speed of response after exposure, automation of the test system and animal ethics.

Ecosystem relevance means that results of biological testing are of importance for the understanding of the effect of a substance or sample to an ecosystem. For environmental risk assessment, extrapolation of the test results to an ecosystem is necessary. Criteria for the ecosystem-relevance of a bioassay are: ecological importance of the test organism, the test parameters and the test-design (see discussion in Peters, 1999).

The test system should be *sensitive* to a broad spectrum of contaminants. Because of the difference of sensitivity of test organisms and as there seems not to exist a species highly sensitive for all substances (Rudolph, 1992; Munawar et al., 1992), a test-set of bioassays with different species and different parameters have to be applied to detect a broad spectrum of toxic substances (Zimmer & Ahlf, 1993).

A further requirement is that the test results should be *reproducible* within a statistical certainty. This is a general requirement for the validity of experimental results. The tests should be *standardised*, which means that the test preparation and procedure is well defined and comprehensible. Standardisation of test methods is a prerequisite for the comparability and reproducibility of the test results.

The term *practicability* includes time, space, personnel, instrumental and economic resources which are necessary to run the test. The test should be *suitable* for the detection of the target substances and appropriate for the aim of the test.

Public perception, implies that the relevance of the test should be understandable. This parameter is of importance, when the test is implemented in regulations and if consequences interfere with stakeholder interests. For some fields of application a *fast response* of the test system following exposure is important, e.g. for early warning systems.

The term *automation* means that the test can be run with more or less sophisticated technical equipment at low personnel costs. Automation is an important parameter e.g. for continuous monitoring. *Animal ethics* should be considered in the development and application of bioassays in general, regardless of the field of application. The consideration of animal ethics are in general required by national law.

The requirement profiles regarding to each field of application are summarised in table 4-2.

Table 4-2: Schematic overview of requirement profiles of bioassays depending on the field of application (Peters, 1999: modified after Nusch, 1991; Rudolph, 1992; Smolka & Weidemann, 1995)

		FIELD OF APPLICATION					
		regulation/ environmental law	effect screening	early warning system	biomonitoring	research/ teaching	hazard/risk assessment
R E Q U I R E M E N T S	ecosystem- relevance	++	+	+	+	+	+++
	sensitivity	+	+	+++	+	-	+
	reproducibility	++	+	+	+	+	+
	standardisation	++	+	-	-	-	-
	practicability	+	++	+	+	-	-
	suitability	+	+	+	+	+	+
	public perception	+	-	-	-	-	+
	speed of response	-	+	+++	-	-	-
	automation	-	+	++	++	-	-
	animal ethics	+	++	+	+	+	+

Legend: +++ decisive + important
 ++ very important - not important

The parameters ecosystem-relevance, reproducibility, suitability and animal ethics are of importance for each field of application, whereas the other six parameters are not necessarily important for all fields of application.

Figure 4-2 summarises in a simplified manner the performances of bioassays with regard to the discussed requirements, differentiated in the level of biological organisation of the test systems.

requirements	eco-system relevance	sensi-tivity	repro-ducibility	standar-disation	practi-cability	suitability	public percep-tion	speed of response	auto-mation	animal ethics
performance	low	high	high	high	high	low	low	high	high	high
biological organisation	molecule									
	cell									
	organ									
	organism									
	population									
	biocoenoses									
ecosystem										
performance	high	low	low	low	low	high	high	low	low	low

Figure 4-2: Performances of bioassays regarding its requirements, differentiated in the level of biological organisation of the test system (Peters, 1999; Steinberg et al., 1999; Fent, 1998; Kanne, 1991).

The performance of test systems towards certain requirements, like ecosystem-relevance and reproducibility is contrary. It should be stressed again, that the diagram gives only a rough overview, for a detailed evaluation see Peters (1999). Whereas most of the requirements can be fulfilled to a certain satisfaction at different levels of biological organisation of test systems, the most challenging requirement is ecosystem-relevance. This is caused by the complexity and the limited understanding of ecosystems. Peters (1999) suggests several criteria for the selection of ecosystem-relevant test-species and test-parameters. The diagram indicates that the ecosystem-relevance of sub-organism test systems is low, because a prediction from effects on the sub-organism level to the ecosystem-level is due to its high degree of extrapolation not possible (Hansen, 1992; Rieß, 1995). Nevertheless, with sub-organism test systems the modes of action of substances can be detected and if basal cytotoxicity (Kristen, 1996) or key functions of living matter (Grimme, 1993) are affected, they can give valuable information for possible consequences on the ecosystem-level.

4.4 Application for Regulatory Purposes

As discussed before (4.2 and 4.3), bioassays are an important tool for the implementation and control of environmental standards in law and regulations. The status of implementation of bioassays and future perspectives with regard to dredged material management in the Netherlands and Germany is given in chapter D 2.4 and D 3.3.

In the following this is complemented by short overviews of other fields of regulatory purposes where bioassays are applied: the notification of chemicals and pesticides, which is required on the national and the EU-level, and whole effluent assessment. The latter is intended to be implemented in the Netherlands and is in practice in Germany for several years.

Bioassays in the notification of chemicals and pesticides

The notification of chemicals and pesticides is regulated on the EU-level by the EU-Directives 79/831/EEC, 91/414/EEC and their amendments. The two directives have the objective in common to protect humans and the environment against potential risks which could arise from marketing new substances or pesticides.

To ensure that this objective is reached, substances newly brought on the market must be notified. Therefore they must be tested according to predetermined criteria for possible hazardous properties and effects. For environmental risk assessments the above mentioned directives also require bioassays. The range of bioassays include sub-organism, mono-species (acute and chronic) and multi-species test systems (detailed overview and evaluation of the bioassays in German implementation of these directives in Peters, 1999). Especially for the notification of pesticides a broad range of bioassays is required, because pesticides are brought into the environment on purpose and are produced to be biologically effective. The gained ecotoxicological data are used for risk assessment and have consequences for labelling, handling, restrictions and prohibitions.

Application of bioassays for whole effluent assessment

In some countries the implementation of water policy regarding to effluents was/is based specifically on the assessment of individual substances or substance groups, called the 'substance-specific approach'. There are many limitations of this substance-specific assessment, such as:

1. a multitude of substances may be present in the effluent, while only a limited number can be analysed;
2. the properties of many substances are often not known, and
3. the effects produced by combinations of substances are also unknown (Tonkes et al., 1998).

Therefore, the characterisation and assessment of effluents with the substance-specific approach is insufficient – a problematic situation for industry, governmental authorities and environmental NGOs.

With the whole effluent assessment (WEA) the limitations of the substance-specific assessment can be overcome and a better understanding of environmental hazards of effluents can be obtained (Tonkes et al., 1998). WEA can be defined as the assessment of the whole effluents by using a range of biological methods or techniques in order to reveal (potential) effects. It focuses on toxicity (acute and chronic), genotoxicity (incl. mutagenicity), bioaccumulation and persistence. Therefore WEA increases the understanding of the combined effect of all known and unknown substances, especially in complex mixtures (OSPAR – PRAM 2000).

WEA can be applied to industrial discharges and effluent discharges of wastewater treatment plants. It can be used to establish priorities in dealing with sources or discharges (Tonkes et al., 1998).

WEA and the OSPAR initial selection of substances in the strategy with regard to hazardous substances (see D 4.2) are both using the same criteria, which are persistence, toxicity and bioaccumulation (PTB). Therefore it was stated on an OSPAR workshop on WEA, that the

objective of the OSPAR strategy with regard to hazardous substances will be served by operationalisation of WEA (OSPAR, 1999). WEA has the potential to be an effective tool e.g.:

- to identify and characterise individual effluents;
- to identify industrial sectors which discharge these effluents;
- to use this tool in the evaluation and development of best available techniques (BAT);
- to develop targets/benchmarks for effluent quality and/or quality of receiving waters (OSPAR – PRAM, 2000).

In this context, the need for research in toxicity identification evaluation (TIE, see 4.6) is stated in order to identify the cause(s) of hazards by backtracking toxicity to causative substances (Tonkes et al., 1998; OSPAR – PRAM, 2000; OSPAR, 1999). This necessity is also stressed by research results in the Rhine delta, where toxic effects could be explained only to a limited extent by the presence of known substances (Hendriks et al., 1994).

The WEA approach is implemented in German environmental law, in the frame of the Water Management Act (Waste Water Ordinance) and the Waste Water Charges Act (see D 3.2.3), for more than ten years. It is used to control the fulfilment of emission standards referring to best available techniques (BAT) for discharges of effluents. Therefore, there are emission limit values – expressed as the Lowest Ineffective Dilution (LID) – for industrial branches stated. WEA is also applied to evaluate immissions, assessing the toxic potentials of wastewaters to the aquatic environment, which is beside chemical and physical analysis the base for wastewater charges. The Ordinance of Waste Water states for the WEA three acute bioassays, the daphnia test, the bacteria bioluminescence test and the acute fish toxicity test. Furthermore, one chronic test, the algae growth inhibition test, and one genotoxicity test (umu-test) are required. All the tests are for freshwater samples. The collection of toxicity data for industrial waste water discharges include over 10,000 samples in 700 industrial plants. The experiences and results of the German WEA approach are evaluated as positive (German Environmental Agency, OSPAR 1999).

In the Netherlands the implementation of the WEA approach as a supplemental tool to the substance-specific approach in the scope of the Fourth National Policy Document on Water Management was originally scheduled for the policy period 1998-2003 (Tonkes et al., 1998). The final implementation is expected in the period 2004-2006 (Tonkes pers. com., 2001). The current status of WEA in the Netherlands is that methods for determining acute toxicity do already exist. Research is currently performed to evaluate the introduction of this biological effect parameter in the permits of the Pollution of Surface Water Act. These are tests with freshwater (algae, crustacea and fish) and salt water (bacteria, algae, crustacea and fish) test organisms. Methods to determine additional parameters are under development, at a stage of method validations. However, the WEA methodology in the Netherlands, especially for chronic tests, bioaccumulation and persistence methods have not been evaluated sufficiently for a complete integration into current policy instruments yet (Tonkes pers. com., 2001). Before proceeding to an actual implementation of WEA, also thorough discussions about the various possibilities and consequences with the parties involved have to be undertaken (Tonkes et al., 1998).

4.5 Chemical Analysis and Bioassays (TIE-like Procedures)

The term TIE (Toxicity Identification Evaluation) refers to TIE protocols established by US-EPA (Mount, 1988). Similar approaches are described in literature where as well the term bioassay-directed chemical analysis (BDCA) was coined (Schuetzle & Lewtas, 1986). These approaches have in common that they combine chemical analysis with bioassays with the aim to identify culprit chemicals, i.e. to identify these substances which mainly contribute to toxic potentials.

Concerning dredged material management, such information could narrow the 'chemical universe' (compare chapter E 3) to a limited number of possibly relevant substances. This could serve as a starting point to conduct substance-oriented case studies towards risk assessment and an analysis of inputs from specific sources and related reduction measures if necessary.

Chemical analysis as well as bioassays are tools used in the assessment of the quality of surface waters including sediments, both having specific advantages (+) and disadvantages (-):

§ Chemical-analytical methods

- + quantitative statements for a limited number of chemicals
- less than 0.5 % of registered compounds are covered by monitoring programmes
- no information about transformation products, bioavailability, synergistic or antagonistic effects

§ Bioassays

- + integrated assessment of effects or toxic potentials of chemical substances
- chemicals responsible for effects or toxic potentials are not identified
- field studies may be needed to verify results from laboratory test systems especially when surrogate organisms or (sub-)cellular test systems are applied (prerequisite for risk assessment)

The combination of non-target chemical analyses and bioassays in TIE-like approaches has the ability to overcome the mentioned disadvantages to some extent. In an iterative process (figure 4-3) environmental samples, e.g. sediments, are extracted and fractionated. Toxic potentials, revealed by the applied bioassays, are linked to causative chemicals by non-target chemical analysis utilising mass spectrometry (HPCL/MS, GC/MS).

Concerning risk assessments at the disposal or relocation sites of dredged material, one has to take into account, that results of TIE-like procedures cannot directly be translated to field situations. One reason is that in TIE-like procedures usually surrogate organisms or *in vitro* toxicity tests are applied in the laboratory, not being representative for the specific ecosystem or covering all relevant specific modes of action under field conditions.

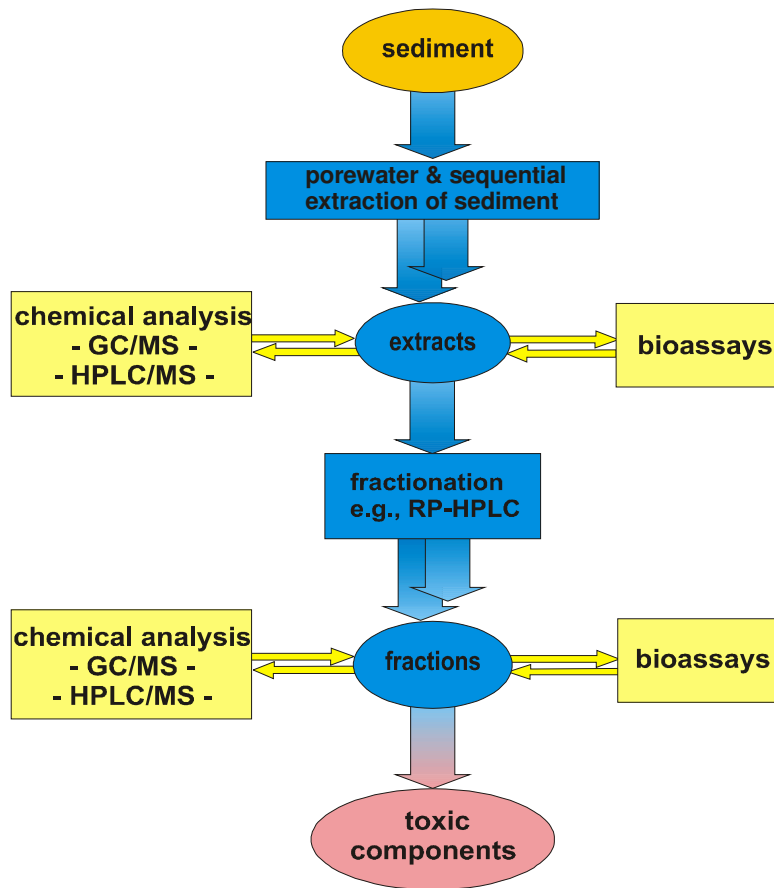


Figure 4-3: Experimental concept of Bioassay-Directed Chemical Analysis (Schmidt, 2000)

The problem of translation of results between laboratory and field conditions is a general one which is as well closely related to the reproducibility of results. With rising complexity of the studied system generally reproducibility decreases. These dependencies are visualised for tools utilised in hazard and risk assessment in figure 4-4 in a schematic manner.

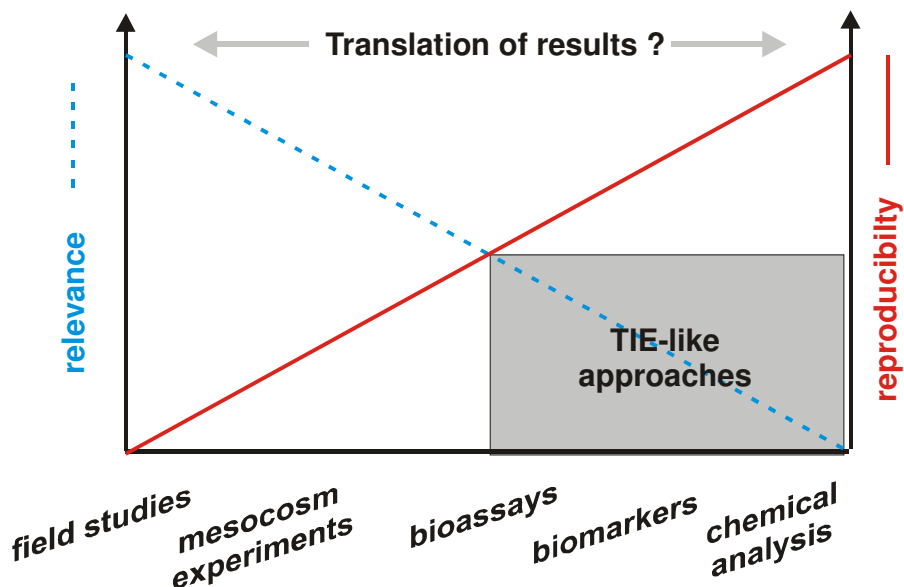


Figure 4-4: Translation of results derived under laboratory conditions and in field studies (Gandrass et al, 2000)

Chemical analysis quantifies concentrations of single substances – standardised and validated methods assumed – at a comparably high reproducibility. However the relevance to field situations with regard to adverse effects to the aquatic ecosystem is low. When assessing e.g. the quality of sediments by combining data from chemical analysis with toxicity data (as far as available from literature for the analysed compounds), important parameters as bioavailability, persistence, bioaccumulation etc. generally cannot be considered or only to a very limited extend. Field studies, e.g. on benthic organisms, have a high relevance towards possible impacts on the ecosystem but have a comparably low reproducibility. In between these two categories fall biomarkers, bioassays and mesocosm studies. Obviously only a combination of these tools/approaches enables a sound risk assessment revealing causative chemicals as well as other stressors. Involved costs generally are higher in more complex systems as mesocosm studies or even field experiments.

There is some consensus that for the sake of being cost-effective, multi-level approaches (so-called tiered approaches) should be followed (Gandrass et al., 1996 & 2000):

- Level I: Limited chemical criteria, limited test battery with bioassays
- Level II: Application of an extended battery of bioassays as well as case studies in order to identify culprit chemicals.

Level I is intended to be used for monitoring or screening purposes. At level II an extended battery of bioassays is applied, covering different modes of actions of chemicals including chronic toxicity. The battery of tests should incorporate different toxicological endpoints as well as organisms from different trophic levels (Zimmer & Ahlf, 1993; SETAC-Europe, 1993). TIE-like procedures should be applied in cases of elevated toxicity, which cannot be explained by the presence of the investigated chemicals.

Recent advances in non-target chemical analysis as well as in bioassays have much improved the applicability of TIE-like procedures. However it has to be stated that low or moderately contaminated materials, e.g. sediments, present still an obstacle when the toxic effect potentials depend on a broad spectrum of chemical compounds at low concentrations (mixture toxicity). Although the contaminants can be identified, they might not be linked any more to their toxic potentials.

4.6 Future perspectives with regard to sediments/dredged material

The capacity of bioassays to assess toxic potentials of chemicals or mixtures of chemicals with regard to specific endpoints (e.g. acute toxicity, genotoxicity) has been utilised in various fields of application including regulatory purposes as pre-market toxicity testing of chemicals including pesticides or whole effluent assessment, thus influencing sediment quality in surface waters indirectly.

The application of bioassays for the assessment of sediment or dredged material quality is advised by international guidelines issued e.g. by the Oslo and Paris, the Helsinki and the London Convention. However, on a national level the implementation of bioassays for the purpose of dredged material management is still under development. In the Netherlands a number of bioassays are evaluated and their implementation is scheduled for 2002.

In the context of sediments/dredged material, bioassays seem to be a promising tool addressing explicitly two issues:

- § Bioassays as additional criteria for the quality of sediments/dredged material might cover chemicals with different modes of action, otherwise overseen relying on a limited set of chemical criteria.
- § An integrated approach, combining bioassays and chemical analysis (so-called TIEs, Toxicity Identification Evaluation) could identify culprit chemicals.

The first can complement the chemical monitoring in a cost-effective manner by investigating integrated toxic effect potentials of the 'cocktail' of substances present in the aquatic environment. The latter not only detects effect potentials but can link them to individual chemicals, which could serve as a basis for more detailed studies and subsequently enable the implementation of specific measures at the sources.

At present the management of dredged materials generally comprises hazard assessment of sediments at the dredging site. Despite the inherent difficulties of conducting risk assessments at the disposal site (the receiving environment, e.g. the North Sea), it should be integrated in future approaches for decision-making frameworks. Further research is needed before implementation.

For the sake of being cost-effective, hazard assessment should be carried out in a multi-level approach:

- Level I: limited chemical criteria, limited test battery with bioassays
- Level II: application of an extended battery of bioassays as well as case studies in order to identify the culprit chemicals

Level II should only be applied for toxic or highly toxic materials where the toxicity can not be explained by the presence of the investigated chemicals. TIE-like procedures can be used to establish links between effect potentials and causative chemicals as well as to distinguish between toxic potentials from man-made and natural compounds (e.g. phytoestrogens).

Additional recommendations, derived during two international workshops, organised as part of this project, dealing with issues related to bioassays and sediments/dredged material

- § At present the application of 3-4 suitable standardised bioassays for acute toxicity including at least one whole sediment test is recommended.
- § Before the implementation, bioassays should be evaluated in a 'research mode' parallel to the currently implemented chemical criteria.
- § Effort should be taken to tackle the interpretation of bioassay results with the long-term goal to integrate the results from different bioassays and possibly even the chemical criteria into one 'yardstick' for the classification of contaminated sediments / dredged material.
- § The development and standardisation of chronic tests and receptor-based assays / biomarkers should be carried out in order to cover other modes of actions and sublethal effects. The latter might in future replace chemical analyses undertaken at high costs (e.g. CALUX assay for chemicals with dioxin-like mode of action).
- § More harmonisation and standardisation of international regulations (guidelines and frameworks) addressing chemical analysis and bioassays as well as hazard/risk assessment is required; while maintaining the integrity of local systems and approaches. An approach which adopts the marine system as the reference point for all other catchment based numbers and ranges might provide a step towards mitigating the issue of uncoordinated regulation, and will also serve to highlight the need for consistency of approaches adopted towards each contaminant.

References

- Fent, K. (1998): Ökotoxikologie. 1. Edition., Georg Thieme Verlag, Stuttgart.
- Forbes, V. E. & Forbes, T. L. (1994): Ecotoxicology in theory and practice. Chapman & Hall, London.
- Gandrass, J., Kurz, J., Salomons, W., eds. (1996): Monitoring Strategies for the Next Century, EUROBASIN Workshop, 27th February 1996, GKSS Research Centre, Geesthacht, Germany.
- Gandrass, J., Salomons, W., Foerstner, U., eds. (2000): Workshop on River Sediments and Related Dredged Material in Europe - Scientific Background from the Viewpoints of Chemistry, Ecotoxicology and Regulations. 3-5 April 2000, GKSS Research Centre. Published by GKSS Research Centre, Geesthacht, Germany, August 2000.
- Grimme, L. H., Rieß, M. H., Manthey, M., Faust, M. & Altenburger, R. (1993): Cell physiological parameters to detect ecotoxicological risks. The Science of the Total Environment Suppl., 741-748.
- Hendriks, A. J., Maas-Diepeveen, J. L., Noordsu, A. & Van der Gaag, M. A. (1994): Monitoring response of XAD-concentrated water in the Rhine delta: a major part of the toxic compounds remains unidentified. Wat. Res. , Vol. 28, No. 3, 581-598.
- Kanne, R. (1991): Aquatische Ökotoxikologie. UWSF - Z.Umweltchem. Ökotox. 3(1), 16-18.
- Klein, W. & Debus, R. (1994): Ökotoxikologie. In: Marquardt, H. & Schäfer, S. G. (Eds.), Lehrbuch der Toxikologie, 748-757, BI-Wiss.-Verlag, Mannheim.
- Kristen, U. (1996): Main features of basal cytotoxicity: sites of toxic action and interaction in the pollen tube cell. ATLA - Alternatives to laboratory animals 24(3), 429-434.
- Mount, D.I. (1988): Methods for Aquatic Toxicity Identifications. EPA/600/3-88-34 to -36. U.S. Environmental Protection Agency.
- Munawar, M., Munawar, I. F., Ross, P. & Dermott, R. (1992): Exploring aquatic ecosystem health: A multi-trophic and an ecosystemic approach. Journal of Aquatic Ecosystem Health 1, 237-252.
- Nusch, E. A. (1991): Ökotoxikologische Testverfahren - Anforderungsprofile in Abhängigkeit vom Anwendungszweck. UWSF - Z. Umweltchem. Ökotox. 3(1), p. 12-15.
- Nusch, E. A. (1996): Ökologische Parameter als Bewertungskriterien in der Ökotoxikologie. In: Deutsche Forschungsgemeinschaft (Eds.), Gewässergütekriterien, Chapter 12, 131-148, VCH Verlagsgesellschaft mbH, Weinheim.
- OSPAR (1999): Outcome of the Workshop on Whole Effluent Assessment, Lylestad 28-29 October 1999.
- OSPAR-PRAM (2000): Whole Effluent Assessment within the Framework of OSPAR, PRAM 00/5/5-E.
- Peters, C. (1999): Die "Belastbarkeit" von Ökotoxizitätstests aus naturwissenschaftlicher und umweltrechtlicher Sicht aufgezeigt an Beispielen des Gewässerschutzes. Diplomarbeit an der Universität Hamburg, Institut für Allgemeine Botanik und Botanischer Garten.
- Plachter, H. (1991): Naturschutz. UTB Gustav Fischer, Stuttgart.

- Pratt, J. R. (1990): Aquatic community response to stress: Prediction and detection of adverse effects. In: Landis, W. G. & Van der Schalie, W. H. (Eds.), Aquatic toxicology and risk assessment, Vol. 13, p. 16-26, ASTM (American Society for Testing and Materials), Philadelphia.
- Rieß, M. H., Manthey, M. & Grimme, L. H. (1995): Zur Bestimmbarkeit des ökotoxischen Schädigungspotentials von Chemikalien. In: Battis, U., Rehbinder, E., & Winter, G. (Eds.), Risikoanalyse und Risikoabwehr, p. 165-202, In: Battis, U., Rehbinder, E. & Winter, G. (Eds.), Umweltrechtliche Studien, Vol. 17, Werner-Verlag GmbH, Düsseldorf.
- Rudolph, P. & Boje, R. (1986): Ökotoxikologie: Grundlagen für die ökotoxikologische Bewertung von Umweltchemikalien nach dem Chemikaliengesetz, Sonderdr. aus: Handbuch des Umweltschutzes, ecomed, Landsberg, München.
- Rudolph, P. (1992): Erkenntnisgrenzen biologischer Testverfahren zur Abbildung ökologischer Wirklichkeiten. In: Steinhäuser, K. G. & Hansen, P.-D (Eds.), Biologische Testverfahren, p. 25-34. In: Leschber, R. & Lahmann, E. (Eds.), Schr.-Reihe Verein WaBoLu, Vol. 89, Gustav-Fischer Verlag, Stuttgart, New York.
- Sachverständigenrat für Umweltfragen (SRU) (1996): Umweltgutachten 1996. BT-Drucks. 13/4108.
- Salomons, W., Turner, K., eds. (2000): Workshop on River Sediments and Related Dredged Material in Europe - River Sediments and Dredged Material as Part of the System Catchment-Coastal Sea: Policy and Regulatory Aspects. 17-19 April 2000, Marine Safety Institute, Rotterdam, The Netherlands. Published by GKSS Research Centre, Geesthacht, Germany, October 2000.
- Schuetzle, D., Lewtas, J. (1986): Bioassay-Directed Chemical Analysis in Environmental Research, Anal. Chem., 58: 1060A-1075A.
- Schmidt, B. (2000): Biotest-geleitete chemische Analyse von Sedimenten. PhD thesis, University of Hamburg. GKSS report 2000/12. GKSS Research Centre, Geesthacht Germany.
- SEATAC-Europe (1993): Guidance Document on Sediment Toxicity Tests and Bioassays for Freshwater and Marine Environments. I.R. Hill, P. Matthiessen, F. Heimbach (Eds.), Workshop on Sediment Toxicity Assessments, 8.-10.11.1993, Renesse, Netherlands.
- Smolka, S. & Weidemann, G. (1995): Eigenschaften ökologischer Systeme und Prognostizierbarkeit von Belastungsfolgen. In: Battis, U., Rehbinder, E., & Winter, G. (Eds.), Risikoanalyse und Risikoabwehr, 203-239, In: Battis, U., Rehbinder, E. & Winter, G. (Eds.), Umweltrechtliche Studien, Vol.17, Werner-Verlag GmbH, Düsseldorf.
- Steinberg, C., Brüggemann, R. & Traunspurger, W. (1999): Ökotoxikologische Beurteilung von Gewässerverunreinigungen. In: Frimmel, F. H. (Eds.), Wasser und Gewässer: Ein Handbuch, Chapter 6, p. 447-505, Spektrum Akad.Verlag, Heidelberg, Berlin.
- Tonkes, M., Pols, H., Warmer, H. & Bakker, V. (1998): Whole Effluent Assessment, RIZA Report 98.034.
- Zimmer, M., Alf, W. (1993): Approaches for assessing sediment quality with ecological and toxicological assays for a development of sediment quality criteria. UBA-FuE 102 04 384/01. Umweltbundesamt, Berlin, Germany.

List of abbreviations

BAT	best available technique
BDCA	bioassay-directed chemical analysis
GC	gas chromatography
HPLC	high performance liquid chromatography
LID	lowest ineffective dilution
MS	mass spectrometry
NGOs	non-governmental organisations
OSPAR	Oslo and Paris Convention
PTB criteria	persistence, toxicity and bioaccumulation
RP-HPLC	reversed phase HPLC
TIE	toxicity identification evaluation
WEA	whole effluent assessment