Dredged Material from the Port of Rotterdam – Interface between Rhine Catchment Area and North Sea –

Workshop Report

River Sediments and Related Dredged Material in Europe

Scientific Background from the Viewpoints of Chemistry, Ecotoxicology and Regulations

GKSS Research Centre, Geesthacht, Germany

3-5 April 2000

The Workshop was organised by the GKSS Research Centre and the Technical University Hamburg-Harburg as part of a project (POR II) for the Rotterdam Municipal Port Management on the current and future contamination of dredged material and related emissions and immissions in the Rhine catchment area.

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Contents

Executive Summary

Dredged material is an important issue from the volumes dredged and its contamination which restricts disposal/relocation options. As part of a project (POR II) on current and future contamination of dredged material and related emissions and immissions in the Rhine catchment area for the Rotterdam Municipal Port Management, the GKSS Research Centre and the Technical University Hamburg-Harburg organised this workshop, attended by invited experts from European countries, from USA and Canada.

The workshop focused on scientific aspects of evaluating and implementing bioassays into decision-making frameworks for dredged material management. Conclusions and recommendations were derived aiming at stakeholders as dredged material managers, e.g. port authorities, river basin managers as well as other national and international authorities and organisations involved in the sediments / dredged material issue. The results of this workshop were presented at a follow-up workshop dealing with policy and regulatory aspects in Rotterdam (17-19 April 2000).

Regulations and guidelines for the hazard assessment of contaminated sediments as well as decision-making frameworks e.g. for the relocation of dredged sediments into the marine environment are not harmonised, neither on an international nor the European level, for some countries not even on the national level.

At present in European countries, the United States and Canada, science-based quality criteria are in use for the assessment of the quality of contaminated sediments. Action levels are derived for management decisions, e.g. permits for relocation into the marine environment, taking into account political considerations (environmental yield / costs ratios). Moreover expert judgement is integrated to a more or less extent into the decision-making processes in different countries.

Current lists of action levels generally consist of chemical criteria for some metals as well as organic contaminants as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. In several countries the use of bioassays as additional criteria for the hazard assessment of contaminated sediments or dredged material is under discussion or already under evaluation. At present a set of a few standardised bioassays for acute toxicity is available.

During the workshop there was consensus that a responsible dredged material management is needed. The main aim should be to reduce emissions from point sources as well as from diffuse sources in the catchment area which would ensure or enable in the long-term

- the reduction of inputs of contaminants via rivers into the marine environment,
- the relocation of dredged material in rivers as well as into the marine environment,
- beneficial uses of dredged material (e.g. in agriculture, habitat creation) and
- cost-effective relocation/use of dredged material

without imposing unacceptable risks to the environment.

At present the management of dredged materials mainly comprises hazard assessment of contaminants at the dredging site. Despite the inherent difficulties of conducting risk assessments at the disposal site (the receiving environment), it should be integrated in future approaches for decision-making frameworks (research demand).

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 (Toxicity Identification Evaluation) approaches are a promising tool 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).

Recommendations on the evaluation and implementation of bioassays as criteria for dredged material management are:

Ø At present the application of 3-4 standardised bioassays for acute toxicity including at least one whole sediment test is recommended.

Ø Before the implementation of these bioassays they should be evaluated in a ‘research mode’ parallel to the currently implemented chemical criteria.

Ø 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.

Ø 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).

As a spin-off from the workshop an initiative for a European Sediment Research Network (SedNet) was launched. It is planned to be driven by stake holder (port authorities, river quality managers) demands and focuses on the dissemination of knowledge, reviewing research needs (problem catalogue) as well as on applied research solving actual problems. It is intended to establish thematic working groups, e.g.:

- “Source Identification Methods“ including TIE-like approaches,
- “From hazard assessment towards risk assessment”.

The latter thematic working group will give a broader platform for the idea – born during the workshop – for a project BIOSAFE (**Bio**logically Based **S**ediment Quality **A**ssessment by **F**ull Scale Field **E**valuation).

1 Introduction

The GKSS Research Centre carries out, on behalf of the Rotterdam Municipal Port Management, and in collaboration with the Institute for Environmental Studies (IVM, Amsterdam), the Institute of Freshwater and Fisheries Ecology (IGB, Berlin) and the Technical University of Hamburg-Harburg (TU-HH, Hamburg) an integrated science-policy study (POR II) on the management of dredged material. Primary aims are to investigate and predict future quality of sediments originating from the Rhine catchment area and current and future policies on regulations with regard to dredged material.

As part of the project this science-oriented workshop had been organised by GKSS and TU-HH, followed by a second workshop held in Rotterdam (17-19 April 2000) organised by GKSS and CSERGE (UK), the latter focussing on policy and regulatory aspects in the scope of river sediments and dredged material as part of the system catchment-coastal sea.

The science-oriented workshop, reported on in this document, was attended by invited experts from European countries, from USA and Canada, presenting lectures and posters during the first public day. During the following two non-public days in two parallel working groups and in the plenum major issues as described in the background paper (Appendix I) were discussed. The workshop agenda is outlined in Appendix II.

The discussion during the workshop focused on scientific aspects of evaluating and implementing bioassays into decision-making frameworks for dredged material management. Conclusions and recommendations should be derived aiming at stakeholders as dredged material managers, e.g. port authorities, river basin managers as well as other national and international authorities and organisations involved in the sediments / dredged material issue. The results of this workshop were presented at the follow-up workshop dealing with policy and regulatory aspects in Rotterdam.

2 Abstracts of lectures and additional abstracts of workshop participants

Bioassays as screening tools for contaminated sediments

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Introduction

Monitoring the various compartments of the environment has traditionally been a field almost exclusively for analytical chemistry. In the early sixties and seventies the main focus of environmental monitoring was on the analysis of persistent and toxic pollutants, such as DDT and their metabolites o,p-DDE and DDD (mainly due to the publication of Rachael Carson's "Silent spring") as well as PCBs (mainly due to their identification in 1966 in fish-eating birds by the Swedish scientist, Soren Jensen). Later on in the late seventies and eighties other persistent organochlorines were added to the list, like Lindane, the drins (dieldrin, aldrin), heavy metals and polycyclic aromatic hydrocarbons (PACs). During these episodes major advances were made in the analytical chemical field, pushing the detection limit from ppm via ppb and ppt down to ppq's. In addition, major achievements were made in the separation and identification of the individual congeners in the complex mixtures of these pollutants in a wide variety of matrices, e.g, from sum PCBs (GC), via 7 major congeners (GC-ECD), to non-ortho(planar), mono-ortho- and di-ortho PCBs (GC-MS) and nowadays the separation and identification of most individual PCB congeners in commercial mixtures by multi-dimensional GC-ECD-MS-MS.

Large data-bases of analytical chemical results from a large variety of matrices, over a wide area of the globe are nowadays available. For many years, the regulation (by law) and assessment of the impact (and measures to be taken) of chemical pollution has (and still is) based on concentrations of specific classes of chemicals in these matrices, with reference to limit values above which actions should be taken. The same is true for dredged sludge and river sediments. For many years the regulatory framework for disposal of dredged material was (and still is) based on analysis of pre-defined classes of persistent chemicals, such as PCBs, heavy metals and mineral oil. By comparing the actual level of sludge contamination with existing limit values, decisions were taken whether the sludge material should be stored in confined disposal, or offshore disposal. The major draw-back of using chemical analytical methodology is that one only obtains results on the level of pre-defined chemicals, mostly the predominant ("high level-low activity") ones.

Low level-high potency chemicals

While this may have been sufficient in the past, and for trend-analysis, recent information on the existence of many other chemicals with relatively high intrinsic biological (and thus potentially toxicological) activity urge for additional measurements to be taken. For example, the recent identification of natural, as well as man-made chemicals with a pseudo-oestrogenic activity have prompted investigators to hypothesize the existence of many “unknown” chemicals in our environment with relatively high biological potency. These so-called “endocrine disruptors” were found to cause toxic effects in both laboratory and wildlife species, and were suggested to also affect human beings (ref). They are present in many environmental compartments, but the majority is believed to be water-borne and may thus be present in both surface/waste water, interstitial water and sludge/sediments.

There are a number of chemical classes that would fit the criterion of “low-level-high activity”, such as the natural and synthetic hormones, man-made hormone disrupting/mimicking compounds, veterinary drugs, certain pesticides and various classes of persistent organo-halogens. In fact, at present it is unclear which and how many different chemical classes would fit the “low level-high activity” profile. This posses an almost impossible task to analytical chemists to come up with adequate strategies for environmental monitoring. Therefore there is an urge to develop alternative/additional (to chemical analysis) screening tools for environmental monitoring, the so-called bioassays, biomarkers approach. This bioassay / biomarker approach in environmental monitoring is promoted further by the increased understanding of the mechanistic principles underlying cause-effect relationships of chemical substances.

Bioassays/biomarkers

There are a wide variety of bioassays/biomarkers developed and reported in literature. Many of those however have been used mainly under laboratory conditions., while only a few have been, or are validated under field conditions. There are bioassays on different levels of biological complexity, e.g., bioassays involving:

- **biomolecules**, such as DNA/RNA and a variety of proteins (receptors, enzymes, carrier proteins), which are mainly used in covalent binding (DNA-adducts), binding-competition (receptors), or inhibition (enzymes) assays
- **cells**: a variety of cell lines, or primary cells from rodents, fish, etc. that are used as e.g., cytotoxicity, proliferation, and reporter gene (CALUX) and biomolecule-induction (vitellogenin) assays
- **tissues and body fluids**: where in vivo responses to contaminant-induced effects are visible as e.g., induced proteins (vitellogenin), altered hormonal levels, hemoglobin adducts
- **whole animal tests**: using sentinel, or surrogate species for toxicity tests, in ecotoxicological setting mostly lethality, and/or reproduction tests

These bioassays, biomarkers are useful and are being used to determine directly (whole animal tests), or estimate with more certainty (cells/biomolecules as marker) the risks for adverse ecotoxicological and environmental effects by (classes of chemicals).

Advantages/limitations of bioassays vs analytical chemical methods

Therefore, if the main focus of environmental monitoring is to determine, or estimate the risk for adverse effects associated with complex chemical contaminants present in a certain matrix (e.g. sediment, sludge) bioassays/biomarkers are the method of choice, because of their superior predictive power. However, the selectivity of some bioassays/biomarkers is quite poor. For example, measuring the lethality of sediment-extracts in whole animal tests of course indicates the total risk/hazard of the complex mixture of contaminants, but is not capable to discriminate between the various classes of culprit chemicals that may be present in the contaminant mixture. On the other hand, if the main focus of environmental monitoring is to measure the levels of certain, pre-defined classes of contaminants and/or on the identification of culprit chemicals the sophisticated analytical chemistry methods (GC-MS, etc) are the best methods to choose, because of their superior selectivity power. Drawback of the analytical chemistry methods is that they are not, directly, capable of predicting the total toxicological impact of the contaminant mixture associated with the matrix (sediment, sludge). Ideally, one would like to use methods that would be capable of determining, or estimating (with sufficient precision) both the concentrations of culprit chemicals and their total toxicological impact. This would allow a well defined handling and management of the matrix of choice, in this case underpin the decision making with respect to storage, or disposal of dredged sludge.

(Pre)screening tools for both exposure and effects

Advances made in the understanding of biological principles of action of chemicals and developments in the field of biotechnology have made it possible to design bioassay methods that may be used for both identification of (classes of) culprit chemicals as well as to indicate the total impact of these classes of chemicals. The principle of action of these methods is based on the way biology deals with identification of bioactive compounds that would (or should) elicit biological responses in the body. The prime candidate target for both identification (based on chemical structure) and for transducing action signals (effects associated with chemical structures) are the so-called “receptor” proteins. Receptors are proteins with specific binding-pockets for defined chemical structures (e.g., hormones, vitamins, essential metals), which transform (after ligand binding) into activated receptor proteins capable to bind to specific regions on the DNA molecule, resulting in the enhanced expression of certain proteins essential for functions such as cell proliferation/differentiation, energy metabolism. Specific hormones, vitamins, that are pivotal for advancing and maintaining almost all essential physiological functions (growth, reproduction, energy metabolism) have their own specific receptors. This biological principle allows the design of chemical (structure) specific signal-transduction switches (reporter genes), which can be used efficiently as bioassays for both exposure and effects. These bioassays are so-called “reporter gene induction assays” that are highly sensitive (capable to respond to pico and femto molar concentrations) and selective (response to certain specific classes of compounds)

Chemical activated luciferase expression (CALUX) assays

The discovery over the last 10 years of similar biological principles of action for xenobiotic compounds has advances the possibilities of using this principle for bioassay development considerably. For example, it was found that the majority of toxic effects caused by the notorious dioxins and dioxin-like PCBs are mediated by the so-called “aryl hydrocarbon receptor (AhR)” pathway. The AhR which resides in the cytosol of cells is capable of binding dioxins and dioxin-like PCBs with high affinity and in a structure-dependent manner, i.e., the most toxic congener (2,3,7,8-tetrachlorodibenzo-p-dioxin) binding the strongest and the least toxic congeners binding the weakest. In addition, there was a good correlation between binding potency and the amount of toxic effects produced in vivo in animals, thereby AhR-binding became a good predictor of toxic potency. Similar receptor-mediated pathways have been discovered for other classes of chemicals, like heavy metals, polycyclic aromatic hydrocarbons (PACs), various classes of endocrine disrupting chemicals (e.g. pseudo-oestrogens), tumor promotion chemicals, etc.

We have used this receptor-based principle to construct and design continuous cell lines (CALUX) that have acquired (through gene transfer technology) a novel property, namely a dioxin-specific switch-on reporter gene (luciferase from the fire fly) which was stably transfected in the cells and thus became an inheritable property of the cells. These DR (dioxin receptor)-CALUX cells will respond to dioxins and related chemicals when added to the culture media by producing high quantities of the fire fly luciferase enzyme, which emits light upon addition of luciferin as a substrate. This CALUX assay was found to be highly sensitive (femtomoles) and selective for dioxin-like, AhR-binding chemicals only. Reproducibility was very good, even at 0,1 pM of dioxins/PCBs the variation coefficient was less than 15% (where HRGC-MS has a variation coefficient of about 30-50%). An additional advantage is that the CALUX read-out is directly the sum total effect of all individual congeners in the mixture (so-called total toxic equivalence), including possible interactive effects (antagonism, synergism). Furthermore, it can be used to measure the bioavailability and biological stability of chemicals in the mixture. It has been used (and at least partly validated) in a wide variety of matrices (food items, milk, body fluids, tissues, sediments, water, air samples, soil) and appears to be a promising tool for screening, or monitoring purposes, and especially suitable for complex mixtures in environmental matrices. Further identification of the culprit chemicals can be advanced by introducing a scheme of toxicity-identification-evaluation (TIE), which in essence is an iteration of bioassay analysis, (sub)fractionation and re-analysis of extracts to determine the “hot” fractions, followed by analytical chemical (e.g. GC-MS) identification of chemical structures. There are many other bioassays that may fulfil the same properties and requirements for environmental monitoring, CALUX is an example of these.

Conclusion

New regulations for handling and managing contaminated sediments and related matrices require the introduction of a set of biological-based tools into the monitoring programme. (Bio)technological advances as well as state of the art science allows for the design nad construction of novel bioassays that may be highly useful as a (pre)screening tool for monitoring purposes, allowing estimation (with sufficient precision) of the total impact (hazard) as well as predicting the (classes of) culprit chemicals. Further identification of defined chemical classes would require an additional procedure of toxicity identification-evaluation (TIE).

Use and usefulness of bioassays to assess sediment quality: A case study of Hamburg Harbour

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Introduction

Contaminated sediments often contain complex mixtures of contaminants, with multiple interactions and diverse biological effects. The chemical basis of bioavailability of contaminants in aquatic systems and those factors that control their toxicity are not well understood. For these reasons, the bioassay approach has been widely used to assess the toxic effects of sediments.

It is important to note, that the response of test organisms vary in their sensitivity to single compounds. Thus, a battery of bioassays is typically used for the detection of potential adverse effects of complex mixtures of contaminants. Toxicity is a dose-related concept, and ecotoxicology of a particular contaminant depends on its fate and how it becomes distributed in ecosystems. In this regard, we have to consider the specific properties of the pollutant in question. Is it mobile, in a geochemical sense, because of aqueous solubility or volatility, or is it associated with solid phases? Different exposure routes require a selection of suitable test organisms. Based on further considerations related to bioassay application, it is obvious that rapid, inexpensive methods are needed.

Strategy

Ecotoxicological testing should be conducted on an appropriate, limited battery of species, endpoints and exposure routes. If this is carefully done and the results are interpreted as an integrative assessment, there is a clear opportunity to prioritize areas of most concern. In a conceptual sediment toxicity exposure model, organisms from different trophic levels can be exposed to toxicants in either of two ways: via porewater or directly from solid-bound contaminants. Elutriates (water extracts) are often used as a surrogate for porewater to assess groundwater hazard. The Elutriate Test was developed as a leaching procedure primarily to determine the mobility of contaminants that are subject to release when solid waste gets in contact with water. The use of elutriates as toxicant solutions has facilitated the testing of standard bioassay organisms, such as *Scenedesmus subtilis* and *Photobacterium phosphoreum*. In some cases, extent and severity of environmental contamination were adequately determined with elutriate testing at hazardous waste sites. However, elutriates represent only a part of multiple contamination, due to the different solubility of each contaminant in water. Water elutriation could underestimate the types and concentrations of bioavailable organic contaminants present.

An additional approach is to use organisms which have contact with the contaminated solids. Tests for whole sediments use organisms such as chironomida or nematoda. It is recommended that within a screening set of tests sediment-living organisms should be preferred. At present, toxicological testing is routinely conducted at the level of the individual organism. Three organism-level measures are required to provide information on the stability of populations and higher levels of organization: survival, growth, and reproduction. If an organism can perform well in all these integrative functions, then it is not adversely affected.

Many experiments have been conducted to evaluate the toxicity of chemicals to chironomides and to identify the pollutants that may adversely affect growth and reproduction of this valuable component of the sediment ecosystem. The 10-day chironomus survival is commonly used as endpoint. The chironomus reproduction test using *Chironomus riparius* may prove to be appropriate to replace the 10-day chironomus survival test. In that case, the test duration would have to be extended to 4 weeks. Therefore, the method is not applicable for screening many samples of a contaminated site. Compared to this, a biotest with nematodes has a couple of advantages: Nematodes are the most abundant and species-rich organisms of the metazoa in sediment. We presented a life cycle-test with sediments, which needs to run for only 72 h. The nematode *Caenorhabditis elegans* has been used in tests for contaminants in liquid phase and in whole sediment samples. The development of first-stage larval worms to the reproductive stage, the number of eggs per adult and the number of offsprings per worm are useful parameters for the ecotoxicological interpretation of test data.

Ideally, sediment toxicity to bacteria should be examined using a representative sediment bacterium and be conducted in the sediment. A contact bioassay using *Bacillus subtilis* or *Arthrobacter globiformis* has been developed using the inhibition of dehydrogenase enzyme activity as endpoint. An advantage is the exposure time of 2 h due to the short generation times of bacteria, which allows toxic effects to be expressed on growing cells for approximately two generations.

Application of a battery of tests to Hamburg Harbor sediments

A three-year study was performed to validate recently developed sediment toxicity tests. The objective of the study was to quantify toxic effects for a sediment quality ranking. About 250 sediment samples were collected and evaluated. More than 90 % of the samples caused toxic effects in the bioassays. Geochemical characterizations of specific areas were compared with bioassay results. We have observed site-specific responses which are related to TBT-contents and particle size distribution. The bioassay results were used to classify the sediment quality by different classification methods.

Fig. 1 shows one example of classification provided by a typical multivariate analysis known as cluster analysis: A classification based on this statistical evaluation is restricted to the type of sediments investigated. Because it is always a relative grouping, the toxicity of sediments from the contaminated Hamburg Harbour range from “lowest measured toxicity” to “highest measured toxicity” – without any information, what “lowest” or “highest” might be compared to different sediments. For a comparison with different data sets, a new cluster analysis would have to be performed, probably resulting in new classes.

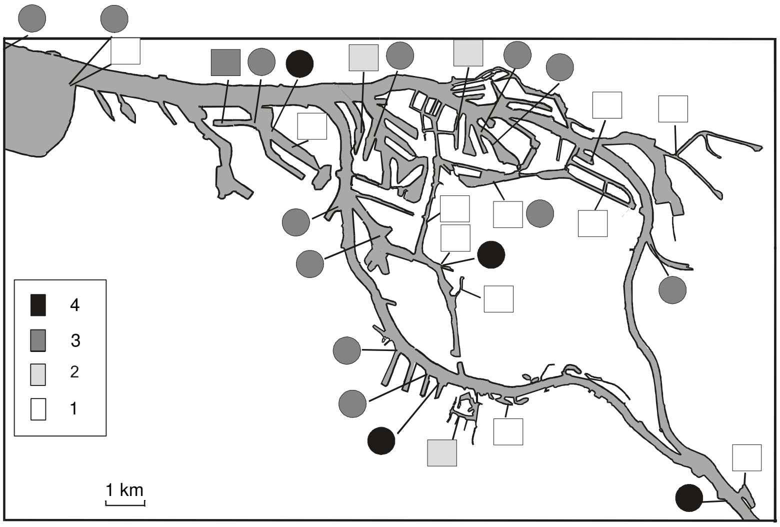

Fig. 1: Ecotoxicological classification of sediment samples from Hamburg Harbour, based on a cluster analysis. Classes range between (1) lowest measured toxicity and (4) highest measured toxicity

The presentation will focus on the interpretation of bioassay results considering variability in space and with time. The use of a fuzzy based expert system for an “absolute” sediment classification will be presented and discussed in detail.

The status and use of bioassays for the assessment of contaminated sediments in the Netherlands

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Introduction

Traditionally the management of contaminated sediments is based on the results of chemical analysis of only a few substances for which generic environmental quality criteria (EQC's) have been derived. Awareness is growing that thus actual, ecotoxicological risk assessment of often complex polluted sediments is not possible because:

- the analysis packet chosen may be inappropriate or incapable of identifying the most harmful substance (e.g. breakdown products);
- bioavailability and combination effects of substances can not be predicted;
- analysis technology is limited, and thus a lot of substances can not yet be detected;
- EQC's are available for only a limited number of substances.

Therefore, complementary use is made of bioassays. Bioassays are laboratory tests in which specific organisms are exposed to contaminated sediment samples or their extracts. Bioassays are performed according to (inter)national standardised guidelines (OECD, ISO, ASTM, OSPAR, NEN, RIKZ SV etc.). If toxic substances are present and bioavailable in toxic concentrations in this sample, then this will result in a clear toxic effect (e.g. mortality) to the test organism. Toxicity can thus be regarded as a sum parameter which, to a great extend, overcomes the mentioned disadvantages of an assessment which is solely based on chemical analysis (1).

This is in short the general Dutch vision on the additional value of using bioassays for the assessment of contaminated sediments. The next part of this paper aims to highlight the current status and developments in the use of bioassays for this purpose in the Netherlands, especially with emphasis on the use as a tool to decide between free or confined disposal of dredged sediment. Furthermore, the possible developments for the coming decade are anticipated.

Present status, use and developments

Status

Two *official* documents are at this moment to a great extend determining the status of sediment bioassays in the Netherlands:

- *Soil Protection Act* (Wet Bodembescherming) (2):

  The "urgent, unless …" disclaimer in the Soil Protection Act states that the need for sediment remediation is less urgent when no actual risks or effects occur at a specific, severely contaminated site. With this in mind a clear, applicable and above all cost-effective ecotoxicological decision framework was developed (3).This decision framework is based on the well known Triad-approach (4) and advises a stepwise application of a selected set of biological techniques, in a recommended sequence:

1 - acute bioassays (pore water tested with: algae, bacteria, crustaceans, rotifers);

2 - chronic bioassays (testing: pore water/water fleas and sediment/midge larvae's);

3 - a bioaccumulation test (aquatic oligochaetes exposed to sediment for 28 days);

4 - field survey (abundance of benthic species and chironomid mentum deformities).

If effects have not been measured after having completed all these steps, it is presumed that the contaminants are less biologically available then expected. It is, therefore unlikely that they cause toxicological effects to the ecosystem. Remediation can thus be regarded as 'not urgent'. If, however, effects are measured, careful considerations are made about the likelihood that effects are due to the concentrations of specific contaminants.

- *Fourth National Policy Document on Integrated Water Management* (4^e Nota Waterhuishouding, abbreviated: NW4) (3):

  In NW4 the government sets out its water management policy intentions for the period 1998-2006. An important statement in this report is: „in the year 2002, an integrated method for the assessment of to be disposed, dredged sediment, comprising biological effect assessment, will be added to the conventional chemical assessment".

  In order to achieve this goal, a set of fresh water and marine sediment bioassays has been selected. For marine sediment testing SOP's have been written (5) (also in English) and the set of bioassays has been round robin tested. The most suitable set of marine sediment bioassays seems to be:

  - Microtox Solid Phase, a 30 minutes bioassay using the bacterium *Vibrio fischeri*;
  - - a 10 day sediment bioassay using the mud shrimp *Corophium volutator*;
  - - a 14 day bioassay using the sea urchin *Echinocardium cordatum*;
  - - DRE Calux, an assay using manipulated cells, modified to detect dioxins.

  This set of assays has been used in 1999 for the monitoring of sediment from ca. 140 Dutch coastal zone and marine harbour locations. This exercise will be repeated this year. Based on the evaluation of these monitoring results, the definitive parameters and assessment criteria for the integrated assessment method will be selected (2001). In 2002 this method will then officially be implemented in the legislation which defines the criteria for free disposal of dredged sediment in open water or confined disposal in large disposal facilities such as the Slufter.

Use

Although the Soil Protection Act gives some ‘legislative space’ for using bioassays as an assessment tool until now bioassays are only voluntary used in the Netherlands to support or modify decisions in the management of contaminated sediments.

During the last two decades ample bioassay experience has been gained by using bioassays for the quality monitoring of contaminated sediments from: the rivers Rhine and Meuse, their sedimentation areas (Delta South and Ketelmeer), marine harbours and from the Dutch coastal zone including the Wadden Sea. The bioassay results were of help to decide to start some sediment remediation pilot projects and the expected ecotoxicological quality improvement is monitored by testing sediment at fixed time periods after the remediation. Bioassays are extensively used in the Dutch Development Programme for Treatment Processes for Contaminated Sediments (POSW) for the assessment of the success of several sediment remediation techniques.

More and more local water quality managers get convinced about the additional value of the use of bioassays for the management of contaminated sediments. At this moment bioassays are already used at local scale for: sediment quality monitoring, assessment of the need and urgency of remediation, the monitoring of remediated sites, the assessment of the disposal of slightly contaminated, (due to hydrological reason) dredged sediment on the banks of brooks and ditches, the assessment of the true threats of sediment pollution to the realisation of ecological water and sediment quality targets (amongst others nature development).

Present developments

The governmental (Rijkswaterstaat) research institutes RIZA (fresh water sediment) and RIKZ (marine sediment) played a leading role in the bioassay developments during the last two decades and still do. They are assisted by a number of non governmental research institutes (a.o. TNO), consultancy firms and universities. Major R&D topics at the moment are:

- *Toxicity Identification and Evaluation* (TIE):

  Bioassays only yield to a minor extend substance specific information. In order to be able to actually use bioassay information for quality management purposes, it can be of great help to know the true cause of toxicity. The US EPA Toxicity Identification Evaluation (TIE) approach provides a promising instrument to unravel cause-effect relations. TIE employs bioassay-directed fractionation, bridging bioassay and chemical-specific analysis, in order to effectively identify toxicants in environmental samples. Some experience has already been gained in the Netherlands with TIE’s on sediment pore water (marine and fresh water) but more research is needed, and in progress, to further develop and implement TIE in sediment assessment strategies.

- *New and complementary bioassays and techniques*:

  A number of Dutch research institutes put a lot of R&D effort in the development of new and complementary bioassays. Topics are: biomarkers (e.g. as quick scan techniques for the detection of toxicity caused by „conventional“ toxicants, for the detection of endocrine disrupting compounds or for detection of pollutants of risk to the DNA integrity). A lot of attention is also paid to the development of: *in situ* or field bioassays (for fresh water and marine sediments), multi species bioassays, micro- and mesocosms (model ecosystems) and to the development of functional bioassays (e.g. aiming at the breakdown of organic material at or in the sediment). Another topic is the concentration of non toxic pore water (e.g. by using XAD resin), thus enhancing the toxicity detection performance of conventional bioassays so that they can also be used to assess slightly contaminated sediment samples.

- *Effect classification*:

  Although (indicative) criteria for classification of bioassay results are available for fresh water (3) and for marine sediments (6), the classification of bioassay results remains an important subject of discussion in the Netherlands. Some workshops have already been held on this issue and new ones are planned. In the mean time dbases have or are been filled with bioassay results of tested Dutch sediments. Statistical (multi variate) analyses of these data helps to further sharpen the criteria for assessment of bioassay results. It is suggested to eventually come to *one*, integrated, effect based, *risk measure*. The PAF (Potentially Affected Fraction of species) concept might be a good starting point for the development of such a risk measure.

Expected developments

Until now the emphasis was on development, selection and performance testing of sediment bioassays. The focus recently changed to the implementation of these assays in water quality management decision frame works. In the near future sediment bioassays will be implemented in the legislation on dredged sediment disposal.

Beside the mentioned present developments in the field of sediment bioassays, the following developments are anticipated for the coming decade (not in chronological order or order of importance):

- *integrative approaches*: sediment will be regarded as an integrated part of the water system, which includes the water column and the banks and shores. Interesting topics, where bioassays could be of use, are for instance the linking of contaminated sediment to:
  - eutrophication problems;
  - the occurrence of algal blooms and thus the cyanotoxin problems;
  - the risks to diary cattle, grazing on the banks where dredged sediment is spread;
  - clearness and odour of surface water (especially in urban areas);
  - *in situ* treatment, e.g.: capping (natural/artificial), toxicant immobilisation via natural present sulphide or addition of charcoal etc.
- Development of Decision Support Systems for specific purposes;
- Development of whole sediment TIE's and TIE's with chronic bioassays;
- Further studying the ecological relevance of bioassay results;
- Further development of strategies for assessment of the environmental impact of the spreading of dredged sediment in open water;

- Growing awareness of the additional value of bioassays and an increase in the use and acceptance of using bioassay information for water quality management purposes;
- International export of gained knowledge/expertise in all the mentioned fields by formation of new thematic networks and co-operation in international projects.

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Use of bioassays in assessing the toxicity of dredged material: Experience in England UK

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Background

The UK Government is assigned to legislation, the 1985 Food and Environment Protection Act Part II (FEPA) preventing the disposal of hazardous materials to sea. The main conditions are to prevent the pollution of the sea by substances that are liable to create hazards to human health, harm to living resources and marine life, to damage or to interfere with other legitimate uses of the sea. In the UK there are approximately 150 applications each year for licenses to deposit dredged material at sea. If successful the licenses are generally valid for one year under FEPA part 2. The total quantity of material licensed for disposal is about 50-60 million tons. The major disposal sites, for example those which receive in excess of one million tons, are close to major ports and estuaries such as the Tyne, Tees, Humber, Thames, Southampton Water, Falmouth, Swansea and the Mersey. The granting of a licence to dispose of dredged material at designated sites around the UK depends on the nature of the material to be deposited and the concentration of contaminants in the dredged material.

There are two classifications for dredged material; capital and maintenance. This classification depends on the geological origin, hydrological cycling, sedimentation patterns and the contamination and frequency of channel or harbour clearance. Capital dredged material is previously undisturbed sediment (clay, chalk, boulder and rocks) and this is material that is not affected by chemicals from anthropogenic activities. The maintenance-dredged material is typically fine-grained silt which accumulates in approach channels to harbours and docks and requires removal and disposal. These materials are often sinks for pollutants and are influenced by anthropogenic compounds such as trace metals, petroleum hydrocarbons or persistent organochlorine compounds. Both the physical and chemical impacts are assessed before a licence is approved and the guidelines are set by the Oslo Commission for the disposal of dredged material. A description of the chemical composition and mineralogical composition of sediments are required from the site proposed for dredging. The Oslo Commission recommends sediment sampling to be conducted to characterise the spatial uniformity of the material in support of the licence.

Under FEPA, MAFF is responsible for licensing of materials in England and Wales. Licenses are issued by the Rural and Marine Protection Division, based on advice provided by the Sea Fisheries Inspectorate and a scientific assessment by staff at the CEFAS Burnham Laboratory. The scientific assessment relies heavily on judgement and experience following a framework and checklist of points, which need to be weighed up in making the assessment. The assessment is not based on a definitive list of steps to be carried out.

The assessment framework consists of information gathering on the quantity of dredged material to be disposed of, sediment type (mud, silt, sand and boulder), chemical nature, method of dredging and disposal, potential for beneficial use, (saltmarsh stabilisation etc), other disposal options and the disposal site characteristics.

The chemical determinands consist of a suite of metals (cadmium, chromium, copper, nickel, lead, mercury, and zinc), organotins, polychlorinated biphenyls (ICES - sum of 7 congeners) and total hydrocarbons. Concentrations of these determinands in the dredged material are used to trigger 'Action Levels' and these are based on the OSPAR guidelines.

Development of Biological tests

It has been realised for a number of years that the reliance on chemical data alone in the assessment framework has weaknesses and that there is an important need to include biological tests. For example chemical analysis measures the total chemical present whereas a biological response reflects the bioavailability of the chemical. A biological test will also provide an assessment of the toxicity of contaminant mixtures, which cannot be achieved through chemical analysis alone. Chemical analysis alone is also very limiting insofar that it is impossible to analyse for every single contaminant that will be present and therefore bioassays have an important role in this respect.

To assess the value of biological tests in the risk assessment process a two-year research programme was carried out at the CEFAS Burnham Laboratory. Several sediment toxicity tests were considered and included methods for whole sediment pore water and sediment elutriate testing. After preliminary trials the following methods were chosen *Arenicola marina* and *Corophium volutator* for whole sediments and *Tisbe battagliai* for elutriate and pore water testing.

> *Arenicola marina* (polycheate) bioassay: Five 1g animals are exposed to ca 2 kg sediment with 5 cm of overlying seawater. After 10 days, the animals are sieved from the sediment and mortality recorded. Throughout the test feeding activity is recorded on a daily basis. A sediment sample is tested in triplicate.
>
> *Corophium volutator* (amphipod) bioassay: Twenty >7 mm animals are exposed to ca. 400 g sediment with 800 ml of overlying water in a 1litre beaker. After 10 days the animals are sieved from the sediment and mortality recorded. A sediment sample is tested using five replicates.
>
> *Tisbe battagliai* (copepod) bioassay: Five juvenile copepods are exposed to 5 ml volumes of elutriate or pore water. Survival is recorded after 24 h and 48 h. Test samples are carried out using four replicates. Pore water is obtained by centrifugation of the sediment sample. Elutriates are obtained by shaking 200 g of sediment with 800 ml of seawater followed by settlement and filtration.

In all of the above tests special importance is given to the measurement of redox, sulphide and ammonia in the test sample at the time of collection and during the test.

Results and conclusions

Bioassays were carried out on eighty dredged material samples over a two-year period. Sediment chemistry was carried out synoptically.

The chemistry data clearly showed that replicate samples were not always homogeneous, some contaminants were one order of magnitude higher. The bioassay results showed that some dredge material samples showed toxicity that would not have been predicted by chemistry alone. In addition, there were occasions when toxic responses were measured with only one of the bioassays which suggests that a battery of techniques should be used in the biological assessment of dredged material. From the data collected in this study a tentative scheme for the inclusion of biological testing in the dredged material risk assessment framework has been proposed but at the present time this has yet to be fully evaluated.

Areas requiring further understanding and development

There are a number of tests being used in different countries and organisations to assess dredged material toxicity and there is a clear need to develop some degree of commonality, in particular with respect to AQC procedures.

There is a clear need to use a battery of tests for assessing dredged material toxicity but what this should consist of at the present time is unclear i.e. species, end points, use of biomarkers etc.

When and how to use biological test results and chemical analytical results in a risk assessment strategy needs to be developed and should not be based solely on statistical significance.

Assessment of dredged material toxicity is in the majority of cases based on acute toxicity test procedures and there is an important place and need to develop a chronic sediment test.

The transfer of persistent compounds through the food chain is an important factor for the licensing of dredged material disposal and will require the development of a bioaccumulation test.

The design and strategy for sampling dredge material to obtain ‘realistic ‘ samples needs to be investigated and should include aspects such as sample storage, depth and method of sampling and statistical aspects.

Effects based testing in the United States dredging program

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Ports and harbors and their associated waterways play a vital role in the U.S. economy, defense, and recreational interests. Maintaining adequate navigation depths in ports and waterways is fundamental to national interests. As sediments are transported and settle in channels and basins, periodic maintenance dredging is required to insure safe passage for shipping. Excavated sediments or dredged materials are removed from channels and transported to other location. There are more than 40,000 km of navigation channels and over 400 harbors in the U.S. Maintenance dredging generates approximately 400 million m^3 of dredged material for disposal annually. About 80% of dredged material is placed in designated sites in the aquatic environment.

Dredging and placement of dredged material are regulated in accordance with a number of environmental statutes including the National Environmental Policy Act of 1969 (NEPA), the Clean Water Act (CWA) of 1972, and the Marine Protection, Research and Sanctuary Act of 1972 (MPRSA). In addition, the U.S. is signatory to the London Convention, which governs the disposal of material in ocean waters. The U.S. Army Corps of Engineers (USACE) has primary responsibility for all aspects of dredging necessary to maintain commercial waterways throughout the United States. Any party (port authority, industrial facility, marina, etc.) wishing to dredge must obtain a permit from the USACE. The permitting process requires a detailed evaluation of the specific dredged material in accordance with regulatory criteria using technical evaluation procedures developed jointly by the U.S. Environmental Protection Agency (USEPA) and USACE (USEPA and USACE 1991, 1998). Because of the complex nature of sediment-contaminant interactions and the fact that contaminated dredged materials can contain complex mixtures of a multitude of toxicants, the USEPA and USACE guidance (1991, 1998) is effects-based. The primary evaluative endpoints are toxicity and bioaccumulation potential of sediment-associated contaminants.

The current guidance manuals (USEPA and USACE 1991, 1998) utilize a tiered approach designed to proceed from simple, cost-effective evaluations, which take advantage of available information, to more complex and costly assessments that provide more detailed answers. An evaluation proceeds through the tiers until necessary and sufficient information is developed to make a decision about how the dredged material should be managed.

Tier I is primarily an evaluation of existing physical, chemical, or biological information. However, in most cases, a more complete chemical characterization of the dredged material will have to be generated. In many cases, a permit decision can be made in Tier I, thus providing a timely and cost-effective regulatory decision. However, in dredged material evaluations involving concerns about contaminants, Tier I will typically indicate that further testing in subsequent tiers is warranted.

A **reference sediment** is used as a basis of comparison in Tiers II, III, and IV. The reference sediment concept implements the regulatory requirement that there be no unacceptable adverse impact. Reference sediment is defined as a sediment that reflects ambient environmental conditions in the disposal site in the absence of dredged material placement. It should be substantially free of contaminants and similar to the grain size of the dredged material and the sediment at the disposal site. Both the reference sediment and the dredged material are tested, and dredged material is evaluated in relation to the reference material. In concept, if the dredged material does not produce an adverse response in test organisms in comparison to the reference sediment, no unacceptable adverse impacts are expected.

Tier II is designed to take advantage of predictive assessment models. Presently, a number of modeling approaches are recommended for use in Tier II, such as the Theoretical Bioaccumulation Potential (TBP) model. The TBP calculation in Tier II is applied as a coarse screen to predict the magnitude of bioaccumulation of nonpolar organic contaminants in the dredged material for comparison with the reference material. For the present, bioaccumulation potential for polar organic compounds, organometals, and metals in dredged material can only be evaluated experimentally in Tiers III or IV. When the TBP for non-polar organic contaminants of concern in the dredged material exceeds the TBP for the reference sediment, or contaminants of concern other than non-polar organics are present in the dredged material, bioaccumulation is evaluated experimentally in Tier III.

TBP can be calculated as

$$TBP = BSAF\ (Cs\ /\ \%TOC)\ \%\ L$$

where TBP is expressed in terms of whole-body wet-weight, Cs = concentration of nonpolar organic chemical in the dredged material or reference sediment; BSAF = biota-sediment accumulation factor; % TOC = total organic carbon content of the dredged material or reference sediment expressed as a decimal fraction; %L = organism lipid content expressed as a decimal fraction of whole-body wet weight. BSAF values for a wide range of compounds and organisms can be found at **http://www.wes.army.mil/el/dots/database.html**.

A numerical model (USEPA and USACE 1998) is also used in Tier II to predict water column concentrations of contaminants at the boundaries of a mixing zone. The model can be used conservatively by assuming that 100% of the contaminants in the dredged material are released to the water column. Alternatively, concentrations in the water column may also be estimated using chemical analysis data from dredged material elutriates. When the concentration of one or more dissolved contaminants of concern are predicted to exceed available Water Quality Standards (WQS), within the mixing zone after 4 h or at any time beyond the boundaries of the mixing zone, management action is required. When standards are not exceeded but synergy among contaminants is a strong possibility or no WQS are available for contaminants present in the dredged material, Tier III toxicity testing is required.

Tier III testing assesses the impact of contaminants in the dredged material on appropriately sensitive organisms to determine if there is the potential for an unacceptable impact at the disposal site. The Tier III assessments include an evaluation of toxicity and bioaccumulation. Where possible, organisms that are representative of those at the disposal sites should be used. Also, exposure routes must be appropriate (e.g., benthic test species must be truly benthic, that is, living on or in the sediment). Presently, Tier III toxicity tests primarily use lethality as the endpoint. Chronic/sublethal tests for sediments are under development; none are considered to be currently suitable for widespread national use.

Water Column Toxicity Tests. Tests to evaluate the impact of dredged materials on the water column involve exposing test organisms to an elutriate dilution series containing both dissolved and suspended components of the dredged material. Three species are recommended for use in water column exposures and should represent different phyla where possible. Candidate test species include mysids (e.g., *Neomysis americana*), cladocerans (e.g., *Ceriodaphnia dubia*), juvenile fish (e.g., *Pimephales promelas*), and bivalve (e.g., *Mytilus edulis*) and echinoderm (e.g., *Strongylocentrotus* sp) larvae.

Elutriates should be prepared using water from the dredging site. Disposal site water or clean water should be used as dilution water. Dredged material and water are combined at a ratio of 4:1 and mixed vigorously. After settling, the supernatant is immediately used for testing. A minimum of 5 replicates per treatment and 10 organisms per replicate are generally recommended. At least 3 concentrations of the dredged material elutriate should be tested (100, 50 and 10%). The recommended test duration is 48-96h. The toxicity data are used to calculate an LC50 expressed as a percent dilution of the elutriate.

If 100% dredged material elutriate toxicity is not statistically higher than the dilution water, the dredged material is not predicted to be acutely toxic to water column organisms. If the concentration of suspended dredged material, after mixing, exceeds 0.01 of the LC50 concentration of dredged material beyond the boundaries of the mixing zone (or 4 h within the zone), the dredged material is predicted to be acutely toxic to water column organisms. In such a case, specific management actions would be required to prevent toxicity beyond the mixing zone.

Benthic Toxicity Tests. Benthic toxicity tests are performed with whole sediment. The use of three sediment-dwelling species of test organisms representing different life history strategies (filter feeder, deposit feeder and burrower) is recommended. No single species would be adequately protective of the broad range of possible chemical contaminants present. Candidate test organisms include amphipods (e.g., *Leptocheirus plumulosus*, *Rhepoxynius abronius*), polychaetes (e.g., *Neanthes arenaceodentata*), mysids (e.g., *Neomysis americana*), commercial shrimp (e.g., *Penaeus* sp.) and grass shrimp (e.g., *Palaemonetes* pugio) for marine sediments, and amphipods (e.g., *Hyalella azteca*), insect larvae (e.g., *Chironomus tentans*), and oligochaetes (e.g., *Tubifex tubifex*) for freshwater sediments. Characteristics to consider for test species selection are: readily available year-round; preferably ingest sediments; tolerate a wide range of grain sizes; give consistent response to toxicants; tolerate laboratory handling; important ecologically and economically. Amphipods are recommended as one of the species tested because of their demonstrated sensitivity to a wide variety of toxicants, their tolerance to a wide range of grain sizes and their ecological relevance for most dredged material disposal sites.

Experimental procedures described in ASTM (1994) and USEPA (1994) are used to conduct amphipod toxicity tests. Tests are typically conducted under static conditions. Renewal of overlying water may be required for certain species or to prevent unacceptable build-up of ammonia or sulfides or low oxygen levels. The standard test duration is 10 d. Dredged material is predicted to be acutely toxic to benthic organisms when mean test mortality is statistically greater than in the reference sediment **and** exceeds mortality in the reference sediment by at least 20%.

Benthic Bioaccumulation. Body burden of chemicals is of concern for both ecological and human health reasons. Bioaccumulation tests are designed to evaluate the potential of benthic organisms to bioaccumulate contaminants of concern from the proposed dredged material. General guidelines for solid-phase bioaccumulation tests are provided in Boese and Lee (1992). The duration of the bioaccumulation test should be sufficient for organisms to approach steady-state tissue residues for non-polar organic compounds. The typical test duration of 28 d is expected to be long enough for most neutral organics (log Kow < 5.5) to approximate steady state tissue concentrations in commonly used test species. Because steady-state concentrations for organic compounds with log Kow higher than 5.5 may not be reached in some species, predictive models may be used to determine steady-state concentrations using data from 28-d exposures (USEPA and USACE 1998).

Test species selected for use in bioaccumulation tests should provide adequate biomass for chemical analysis, preferably ingest sediments and survive in the dredged material and control and reference equally well. In addition, inability to metabolize some types of organic compounds (e.g., PAHs) is desirable in test species. At least two different species should be utilized. Candidate species include polychaetes (e.g., *Neanthes arenaceodentata*) and bivalves (e.g., *Macoma nasuta*) for marine sediments, and oligochaetes (e.g., *Lumbriculus variegatus*) and insect larvae (e.g., *Hexagenia limbata*) for freshwater sediments.

Contaminants of concern in tissues of benthic organisms following laboratory exposure to dredged material are first compared to applicable Food and Drug Administration (FDA) Tolerance Levels for Poisonous and Deleterious Substances in Fish and Shellfish. The FDA levels are based on human-health and economic considerations, but do not indicate the potential for environmental impact on organisms exposed to the dredged material. If tissue concentrations of one or more contaminants are statistically higher than the FDA levels, the benthic material is predicted to result in unacceptable benthic bioaccumulation of contaminants. If FDA levels are not exceeded, tissue concentrations following exposure to the dredged material are compared to tissue concentrations in organisms similarly exposed to reference sediment. When tissue concentrations in organisms exposed to dredged material statistically exceeds those of organisms exposed to the reference material, other factors are considered, such as the magnitude of the difference, the toxicological importance of the contaminants, and the likelihood of biomagnification. The web-based Environmental Residue-Effect Database (ERED, **http://www.wes.army.mil/el/ered/index.html**) contains valuable information for ecological interpretation of bioaccumulation data. ERED is a compilation of data, taken from the literature, where biological effects (e.g., reduced survival, growth, etc.) and tissue contaminant concentrations were simultaneously measured in the same organism.

Tier IV involves case-specific, state-of-the art testing for toxicity and bioaccumulation and is to be used on a case-by-case basis only when lower tiered testing is judged to be insufficient to make decisions (e.g., when evidence is conflicting). Tier IV evaluations include benthic and water column chronic/sublethal toxicity testing, steady state bioaccumulation testing and formal risk assessment.

Current research conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, Mississippi, is designed to enhance the dredging material evaluation process. Standard protocols for conducting chronic sublethal sediment toxicity tests with the estuarine amphipod *Leptocheirus plumulosus* and the polychaete *Neanthes arenaceodentata* are being developed. The relative sensitivity of the acute and chronic tests with *L. plumulosus* is being compared. In addition, the relationship between sediment concentration, bioaccumulation and biological effects is being investigated for a variety of organic compounds. Critical body residues for non-polar organic compounds is being determined for a variety of invertebrate species and will provide valuable information for the development of environmentally realistic guidelines for interpreting bioaccumulation studies, as well as for comparing test species sensitivity. Non-laboratory work includes the development of risk assessment methods for dredged materials.

Detailed information on the U.S. dredging research can be obtained from the Dredging Operations Technical Support (DOTS), the Dredging Operations Environmental Research (DOER) and the Long-term Effects of Dredging Operations (LEDO) programs. Links to the DOER and LEDO homepages, as well as online access to Testing Manuals and other relevant publications are available from the DOTS home page
(**http://www.wes.army.mil/el/dots/dots.html**).

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Setting toxicity criteria using multiple test endpoints: A comparison of multivariate and ranking methods

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Canadian Approach to Contaminated Sediments in the Laurentian Great Lakes

Sediment quality guidelines (SQGs) in Canada are currently based solely on contaminant concentrations. The formal procedure for deriving national SQGs for freshwater and marine systems was developed by the Canadian Council of Ministers of the Environment (CCME 1995) and was intended to provide broad protection of the functioning of healthy aquatic ecosystems, the procedure is similar to the approach of the National Status and Trends Program (NSTP) of the US National Oceanic and Atmospheric Administration (Long and MacDonald 1992).

The NSTP approach is a co-occurrence method, in which SQG values are calculated based on associations of a range of contaminant concentrations with observed biological responses. Canadian SQGs are derived on chemical-by-chemical basis by a series of steps:

1. Compilation of data from
   - field studies with synoptically measured contaminant concentrations and biological effects;
   - equilibrium partitioning models for contaminants in sediment;
   - SQGs for other jurisdictions; and
   - spiked-sediment toxicity tests.
2. Evaluation and screening of the data to produce a biological effects database for sediments.
3. Generation of tables that summarize effect and no effect associations.
4. Calculation of SQGs:
   - TEL = “threshold effect level” = geometric mean of lower 15th percentile concentration of the effect data set and the 50th percentile concentration of the no-effect data set;
   - PEL = “probable effect level” = geometric mean of lower 50th percentile concentration of the effect data set and the 85th percentile concentration of the no-effect data set.

The TEL represents the concentration of a contaminant below which adverse biological effects are expected to occur rarely, whereas the PEL represents the concentration above which effects are expected to occur frequently. Development of national SQGs is ongoing. To date there are guidelines for 24 substances: 9 metals, 6 polycyclic aromatic hydrocarbon compounds, 8 pesticides, and total polychlorinated biphenyls (Environment Canada 1995).

In recognition of their limits in application to sediment assessment, SQGs are recommended for use as *benchmarks* in a stepped “decision-tree” or “multiple-lines-of-evidence” framework. In such assessments, exceedences of SQGs are indicative of *possible* negative effects, which are then verified by further bioassessment. Although there is presently no standardized assessment framework for Canada (Zarull et al. 1999), several multiple-lines-of-evidence approaches have been developed, including the “sediment quality triad” (Long and Chapman 1985; Chapman 1990) and the “BEAST” methodology (Reynoldson et al. 1995).

Evolution of Sediment Quality Guidelines

Ideally, SQGs should be developed from controlled dose-response experiments and confirmatory field observational studies. Environmental factors that modify toxicity should also be incorporated to improve predictions of toxicity. However, the reality of achieving such an objective given the complexity of sediment-contaminant interactions is questionable, and to date there is not adequate scientific information available to support such an approach. Current SQGs are not sufficient for assessment of ecological conditions or to guide remediation because they do not address:

- contaminants for which guidelines have not been developed;
- unmeasured contaminants; or
- site-specific conditions (modifying or mitigating factors; interactions with other contaminants).

Furthermore, they are based on single data sets using the same data to develop guidelines for individual chemicals without knowledge of which contaminant is producing observed effects.

Since the underlying reason for chemically based SQGs is the protection of the structure and function of aquatic biota, many workers (e.g., Reynoldson and Zarull 1993) have argued for an ecological approach to developing sediment guidelines. These include assessing structural biological attributes, such as benthic invertebrate community composition and density, and functional attributes, such as sediment toxicity.

The approach of Reynoldson et al. (1995), which is derived from methods developed in the UK (Wright et al. 1984), was developed for assessing nearshore sediments of the Laurentian Great Lakes. It involves sampling a large number of uncontaminated (reference) sites throughout the region to define the normal range of conditions for undisturbed sediment. In other words, it establishes what a “healthy” benthic condition should look like. The approach allows appropriate site-specific biological objectives to be set for ecosystems from measured habitat characteristics, and provides a meaningful reference for identifying anthropogenically-induced degradation.

Application of the approach involves the collection and analyses of data on selected physico-chemical habitat descriptors, benthic invertebrate community structure and sediment toxicity. The discussion below focuses on the toxicological component of the decision making framework, and compares methods for combining multiple measures of toxicity to establish assessment criteria.

Integrating Multiple Measures of Toxicity

Laboratory toxicity testing is frequently used for assessing field collected sediment, and an array of test species, endpoints and protocols have been proposed. In almost all cases a number of tests are used in assessing sediment toxicity as no single species or endpoint is universally appropriate, sensitive or practical. While there is general agreement that a battery of tests is required there is little guidance or advice proposed on either setting effect sizes for individual test endpoints or on integrating multiple test endpoints into what is a binary decision on whether a sediment is assessed as being toxic. A large sampling programme in the Great Lakes measured the response range for ten test endpoints (growth, survival, and reproduction) in four benthic invertebrate species (*Chironomus riparius, Hexagenia limbata, Hyalella azteca*, and *Tubifex tubifex*) across a range of uncontaminated sites (170-220) representing an array of sediment conditions. From these data it was possible to describe the normal response range for each test endpoint and establish numeric criteria.

On a single endpoint basis, sediment toxicity (or enrichment in the case of growth) can be identified when the bioassay response to a test sample falls outside the normal range (e.g., ± 2 SD from the mean) for the reference sites. For the range of sediments examined, there was little variability in the bioassay responses. Thus single criteria values could be derived for each endpoint that did not require adjustment for effects of modifying habitat factors. Incorporation of all the endpoints into a single, more comprehensive and interpretable assessment criterion, requires several decisions to be made on the relative scaling and weighting of the individual endpoints. Three methods were examined here for integrating the ten test endpoints.

Two methods used a scoring system. In these cases each endpoint was assigned a score of 1, 2 or 3 representing a non-toxic, possibly toxic or a toxic response, respectively, in a test sediment. For the first method, median scores for the test sites were calculated but were overridden by a lethal endpoint score that exceeds the median. In the second method, scores are summed for each of the lethal and sublethal sets of endpoints.

The third method used ordination, a multivariate statistical procedure that "compresses" the information carried by a series of variables into a smaller number of synthetic variables. In this case, toxicity responses in 10 dimensions were transformed into 2 dimensions. Both the reference and test sites were involved, and were presented in bivariate plots. The degree of difference between the test site and the normal range for the reference sites was used as a measure of the sediment toxicity. A comparison of the methods showed the multivariate technique to be the most sensitive and informative because it:

- minimises effects of correlated variables;
- includes quantitative information;
- weights endpoints appropriately;
- allows determination of relationships with abiotic sediment attributes.

Integration of multiple measures of toxicity into a single criterion simplifies the identification of toxic sediments. In doing so, the above method incorporates information from all endpoints. Together with an assessment of *in situ* benthic invertebrate communities (using the same reference condition approach), sediment toxicity tests represent ecological responses to sediment conditions. As such, they are important supplements to the usually analyzed contaminant data for the development of SQGs.

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Selection and use of marine toxicity assays to assess the quality of dredged sediments

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ABSTRACT

Sediment quality criteria for marine sediments, and more in particular dredge disposal sediments, have an important role in the environmental management of contaminated sediments and dredging spoils. However, the use of contamination levels based on chemical analyses alone are inappropriate to assess the impact of the contamination on the ecosystem. The importance of the contribution of toxicity testing, when incorporated in sediment quality schemes, has already been recognised and recommended by a number of international fora (Oslo and Paris Commissions, International Council for the Exploration of the Sea). The aim of the current study was to select and assess an ecotoxicological test battery for the routine characterisation of marine sediments. Six contaminated and one reference sediment were used in this study. Acute bioassays were performed on porewaters using the following organisms: the oyster larvae C. gigas, the copepod A. tonsa, the rotifer B. plicatilis, the algae P. tricornutum, the bacteria V. fischeri (Microtox^R) and the mysid M. bahia. Acute or chronic sediment contact tests were performed with the mysid M. bahia, the lugworm A. marina and the amphipod C. volutator. Based on the toxicity results three acute pore-water toxicity tests (Microtox^R, A. tonsa and B. plicatilis) and one whole sediment toxicity test (C. volutator) were selected for the test battery. Only with the latter assay was a discrimination possible between various 'grey zone' sediments (i.e. contamination levels between target and limit values as proposed in currently chemical analysis based regulations) leading to the identification of toxic and non-toxic sediments.

1. INTRODUCTION

The degree of contamination causing adverse effects on an ecosystem's structure and function cannot be determined, based solely on chemical analysis. Factors such as bioavailability and synergistic/ antagonistic interactions of contaminants demand an effect-based assessment of environmental contamination. This implies the application of bioassays using test species which are representative for the investigated biotic community. Some international organisations are currently focussing on the ecotoxicological evaluation of marine sediments and dredging spoils (Oslo and Paris Conventions for the Prevention of Marine Pollution, Society of Environmental

Toxicology and Chemistry, Joint Assessment and Monitoring Programme, International Council for the Exploration of the Sea). Until now there is no international harmonisation as to the preferred toxicity tests. Moreover, current legislation in most European countries do not require ecotoxicological assessments of sediments and only impose target- and limit values for a broad range of contaminants (e.g. heavy metals, TBT, PAHs, PCBs, organochlorines, pesticides such as DDT, aldrin, dieldrin, lindane,..) (OSPARCOM, 1998).

Di Toro (1990) stated that pore water is the primary exposure medium in the toxicity of sediments towards benthic organisms. However, the use of porewater in the evaluation of sediments has been criticised as extraction procedures may alter the bioavailability of some contaminants. The JAMP Guidelines for general biological effects monitoring suggests the use of the oyster embryo test with *Crassostrea gigas* for porewater testing (Thain, 1991). The harpacticoid copepods *Tisbe battagliai* (Williams, 1992) and *Nicotra spinipes* (Dave *et al.*, 1993) and the polychaete *Dinophilus gyrociliatus* (Carr *et al.*, 1989) can be used for specific applications (e.g. low salinities and chronic endpoints) (JAMP, 1998). Other assays used for the routine assessment of porewaters include methods with the sea urchin *Arbacia punctulata* (Carr and Chapman, 1992, Burgess *et al.*, 1993), fish embryos, algae (*Phaeodactylum tricornutum*), the mysid shrimp *Mysidopsis bahia* (renamed *Americamysis bahia*, Price *et al.*, 1994)) and with *Vibrio fischeri* (Microtox^{R} bioluminescence assay). It should be emphasized that there are, at present, no international agreed protocols/guidelines for extracting and testing marine porewaters.

Whole sediment testing allows the evaluation of environmental effects under conditions which are (more) representative for *in situ* conditions. Possible test organisms mentioned in JAMP are: the amphipod *Corophium volutator*, the lugworm *Arenicola marina* (Thain and Bifield, 1993) and the echinoderm *Echinocardium cordatum* (Bowmer, 1993). For *C. volutator*, an internationally agreed protocol has been published by OSPAR (1995). Other frequently used methods include the whole sediment assay with the amphipods *Grandidierella japonica* (American Society for Testing and Materials, 1992; Environment Canada, 1992; Carr, 1993) and *Ampelisca abdita* (ASTM, 1992), the mollusc *Abra alba* (Stromgren *et al.*, 1993) and the polychaetes *Nereis* spec. and *Neanthes* spec. (McLeese *et al.*, 1982; Johns *et al.*, 1991; Dillon *et al.*, 1993). No internationally accepted protocols for chronic whole sediment testing are presently available. This represent a major short-coming in current testing schemes and hampers the evaluation of long-term effects of sediment–associated contaminants.

The aim of this study is to select and assess of a battery of ecotoxicity tests which can be applied in routine for evaluating marine sediments. It was of particular importance to ensure that the selected battery had a sufficient “discriminative power”, allowing to differentiate between toxic and non-toxic sediments belonging to the 'grey zone'. Grey zone sediments are defined as those for which one or more chemical parameters exceed the target value, but for which all parameters are lower than the limit value (OSPARCOM, 1998). The selection of the test organisms is based on ecological relevance, use in other sediment evaluation studies and cost-effectiveness.

2. MATERIALS AND METHODS

2.1. Sediment sampling

Six marine sediments were selected to represent a wide range of contaminant level and –type (S1 to S6). The sediment selected as reference sediment (R1) contained no or minor concentrations of the analysed contaminants. All samples originated from Belgian coastal waters or from harbours. Bulk sediment was collected with a Van Veen-grab and transported in 10 L high density polyethylene containers with minimal headspace and stored at 4°C until processed for use in the toxicity tests. Porewater for both chemical and toxicity analysis was separated from the solid phase by centrifugation (4000 rpm; 15 min) and filtration (0.45 µm). Porewater was stored at 4°C in glass bottles for maximum 48 hours. For whole-phase sediment testing, seawater was poured gently on top of the homogenised sediment (volumetric 4:1 seawater-sediment ratio) 24 hours prior to the start of the bioassays. Temperature and oxygen-concentration were monitored during the exposure period and aeration was applied when necessary (i.e. < 40% oxygen saturation).

2.2.Chemical analysis

The following parameters were analysed for the seven sediment samples: pH, Eh, ammonium, dry weight (DW) at 105 °C, grain size distribution expressed as % of the total DW (16, 25, 63, 2000, > 2000 µm), organic DW as % of the total DW, heavy metals in mg/kg DW (Cd, Cr, Cu, Ni, Pb, Zn, Hg, As), mineral oils in mg/kg DW, PCBs (PCB 28, 52, 101, 118, 153, 138, 180) in µg/kg DW and PAHs (6 according to Borneff) in mg /kg DW.

2.3.Toxicity tests

Table 1 summarizes the acute porewater toxicity tests which were used. A detailed description of the used toxicity tests is beyond the scope of this paper. The Microtox^R assay was conducted following the 100% procedure outlined by Microbics Co-operation (Microbics, 1992). The 72h algal growth inhibition test was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 201 (1984). The 24h-oyster larvae test with *C. gigas* was conducted using the test procedure described by Thain (1991). The 24h acute cyst-based toxicity test with the rotifer *B. plicatilis* was performed according the procedure described by Snell and Persoone (1989). The 48h-test with the copepod *A. tonsa* was based on the (draft) standard method ISO/DIS 14669 (1997). Finally, the 48h-assay with the mysid shrimp *M. bahia* was performed according the methods developed by the U.S. Environmental Protection Agency (1985). No chronic porewater tests were performed due to some inherent practical problems (chemical instability, large volumes of porewater needed).

Table 1: Porewater bioassays used for the evaluation of the marine sediments

Test species	Test duration	Endpoint
Vibrio fischeri (Microtox^R)	15 min	Bioluminescence
Phaeodactylum tricornutum	72 h	Growth
Crassostrea gigas	24 h	Development
Brachionus plicatilis	24 h	Mortality
Acartia tonsa	48 h	Mortality
Mysidopsis bahia	96 h	Mortality

The whole sediment bioassays used in the current study are noted in Table 2. Some of these assays are described and standardized by international organisations: the test procedure with the oyster larvae *C. gigas* is described in detail in ASTM Guideline E724 (ASTM, 1997). The 10 day whole sediment assay with the amphipod *C. volutator* is based on the method described by Environment Canada (1992). The other test procedures are less standardized or are still under development *C. volutator and A. marina* were field collected from non-polluted areas (reference points). All other test organisms (except the cyst-based assay with *B. plicatilis* and *C. gigas* which were purchased) were cultured successfully in the laboratory. Depending on the organism and test procedure, the whole sediment assays were either static, semi-static or flow-through. More details are given in the cited references.

Table 2: Whole sediment bioassays used for the evaluation of the marine sediments

Test species	Test duration	Endpoint
Crassostrea gigas	48 h	Development
Arenicola marina	10 d	Mortality
	21 d	Mortality
Mysidopsis bahia	14 d	Mortality and growth
Corophium volutator	10 d	Mortality
	28 d	Mortality and growth

2.4.Data analysis

In tests where mortality exceeded 50% the LC_{50} values and corresponding 95% confidence intervals (CI) were calculated using the moving average method (Stephan, 1977). The LC_{50} was estimated by the binomial method for those assays which did not result in partial mortality scores. The EC_{50}s resulting from the Microtox assay were calculated using linear regression as described by Microbics (1992). The EC_{50} based on biomass measurements for the algal growth inhibition test was calculated according to OECD 201 (1984). Effect (lethal) concentrations of the porewater assays were transformed into Toxic Units (T.U.), according to Sprague and Ramsay (1965):

$$T.U. = 100 / L(E)C_{50}$$

Data for percentage survival/growth were arcsine square root transformed and then tested for normality and homoscedasticity using the Kolmogorov-Smirnov and Bartlett tests. Arcsine square root transformed data fulfilled assumptions of parametric statistics so significant differences of mean survival/growth were made using a one-way analysis of variance (ANOVA) in combination with Duncan multiple range test (Sokal and Rohlf, 1981).

3. RESULTS AND DISCUSSION

3.1 Chemical analysis

Table 3 summarizes the concentrations of the different contaminants found in the seven sediment samples.

For the reference sediment and sediment S1 none of the measured parameters exceeded the Sediment Quality Criteria target value. In S3, S5 and S6 target values are exceeded for mercury, zinc and copper. For two sites (S2 and S4) the limit value was exceeded for one contaminant.

Table 3: Chemical analysis results of seven marine sediments:. Values according to the Belgian Sediment Quality Criteria

µg/kg DW	**R1**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Hg	0.15	0.27	0.81	0.53	0.47	0.38	0.27
Cd	0.2	0.5	7.1	1	0.7	0.9	0.4
Pb	20	30	108	58	39	40	28
Zn	61	94	400	274	162	181	107
Ni	10	16	26	20	17	16	14
As	9.6	12.5	13.6	14.6	12.9	10.4	11.6
Cr	29	41	85	54	46	46	38
Cu	9	14	138	77	49	27	29
Min. Oil (in mg/kg)	27	37	633	1220	425	438	156
PAH's (16) (in mg/kg)	0.31	1.3	5.631	30.3	247.6	1.9	13.8
PCB's (7)	1.1	4.2	78.7	22.8	19.7	24.5	10.4

(light grey) = between target and limit value (according to the Belgian Sediment Quality Criteria)

(dark grey) = above the limit value (according to the Belgian Sediment Quality Critera)

The sediments can be divided into three groups:

A: contaminants below target value: R1 and S1

B: at least one contaminant in the ‘grey zone’: S3, S5, S6.

C: at least one contaminant above limit value: S2 and S4.

3.2. Ecotoxicological evaluation

Table 4 summarizes the acute toxicity test results obtained with the porewaters.

Table 4: Ecotoxicity of porewaters originating from the 7 tested sediments. Results expressed in T.U.; NT = no toxicity detected

	R1	S1	S2	S3	S4	S5	S6
V. *fischeri*	NT	NT	NT	NT	0.8	NT	NT
P. tricornutum	NT	NT	1.8	1.7	1.4	> 16	1.1
B. plicatilis	NT	NT	NT	NT	NT	NT	NT
C. gigas	12.2	5.8	12.0	11.7	10	28.1	> 32
A. tonsa	7	2.4	3.8	3.4	4	14.7	5
M. bahia	4.4	7.8	4.5	3.8	5.4	8.6	4.4

For each of the sediments a toxic signal was detected with at least one of the toxicity tests. With exception of S4 no toxicity was detected with the rotifer *B. plicatilis* and *V. fischeri*. With the algae *P. tricornutum*, growth inhibition was measured in all porewaters, except in those from the two non-contaminated sites (R1 and S1). In these porewaters normal growth was measured. All porewaters, including R1 and S1, caused moderate to severe acute effects for the oyster larvae *C. gigas*, the copepod *A. tonsa* and the mysid shrimp *M. bahia*. The observed toxicity, however, can be explained by the high ammonia concentrations in the porewaters (25-100 mg/L). Ammonium is a natural, biological compound of which free ammonia (NH_3) is the most toxic form. Ammonia as toxicity causing agent was confirmed using the Toxicity Identification Evaluation methods with the copepod *A. tonsa* (data not shown). *P. tricornutum* was much less sensitive towards elevated ammonia concentrations. Indeed, although the porewater of S1 contained an NH_4^+-concentration of 55 mg/L, no toxicity was noted. A still higher ammonia concentration was measured in porewater S4 (110 mg/L). These results could suggest that the presence of contaminants other than ammonia are responsible for the observed effects on *P. tricornutum*. It should be emphasized that colloidal particles were present in the highest algal test concentrations. These particles may have interfered with the algal density measurements, possibly causing false positives. The high sensitivity of *C. gigas*, *A. tonsa* and *M. bahia* towards ammonia limits their toxicity detecting capacity for other contaminants. The application of ammonia-removal techniques as suggested by some authors may resolve this problem (Stronkhorst *et al.*, 1996). Care should be taken when applying these methods as they can alter the bioavailability/toxicity of contaminants. The results of the whole sediment bioassays with *M. bahia* and *C. volutator* are presented in Table 5.

Table 5: Results of whole sediment bioassays with *M. bahia* and *C. volutator*. Mortality is expressed in % ± SD, growth in mg/organism ± SD. Significant effects ($p<0.05$) compared to R1 are indicated in bold; / = no data available.

	R1	S1	S2	S3	S4	S5	S6
Mysidopsis bahia							
Mortality	16.7	16.7	10.0	0	13.4	26.7	10.0
(14 d)	± 11.5	± 5.8	± 17.3		± 23.1	± 46.2	± 10.0
Growth	0.247	0.269	0.287	0.232	0.235	0.301	0.341
(21 d)	± 0.049	± 0.075	± 0.073	± 0.012	± 0.091	± 0.042	± 0.029
Corophium volutator							
Mortality	13.7	**81.1**	3.3	0	**100**	**77.8**	40.9
(10 d)	± 2.9	**± 11.7**	± 5.8			**± 22.2**	± 32.4
Growth	0.667	**0.189**	**0.422**	**0.319**	/	/	**0.419**
(10 d)	± 0.156	**± 0.002**	**± 0.120**	**± 0.025**			**± 0.059**
Mortality	33.3	60	8.2	6.7	**100**	**100**	33.3
(28d)	± 15.3	± 45.8	± 4.9	± 5.8			± 20.8
Growth	0.883	0.928	0.643	0.531	/	/	0.670
(28 d)	± 0.296	± 0.002	± 0.104	± 0.233			± 0.145

None of the sediments caused a significant effect on the mysid shrimp *M. bahia* (both endpoints). *M. bahia* is an epibenthic organism and can avoid contact with the contaminated sediment. In order to stimulate contact and possible uptake of sediment particles, the organisms were only fed three times during the test period. The impact of this restricted feeding regime resulted in a complete inhibition of reproduction. With *C. volutator* effects on mortality or growth were detected in all tests, except those with R1 sediment. Although none of the measured chemical parameters exceeded the target values for sediment S1 a high mortality was noted. A possible explanation for this phenomenon is the presence of a toxic contaminant other than the measured compounds emphazising once more the importance of the use of ecotoxicological assays for the characterisation of sediment quality. Although significant growth reduction was found for S2, S3 and S6 tests, no significant mortality was observed for these sediments. Prolongation of the test period to 28 days resulted in more variation between the replicates and the results could not be used to yield more information.

The tested sediments did not cause significant effects on *C. gigas* (data not shown). This test which is a combination of a whole sediment, pore water and elutriate assay (large dilution of sediment in seawater: 18 g/900mL) cannot be considered as a conventional whole sediment assay. The large sediment dilution, combined with the free-swimming character of the oyster larvae, may be the main reason for the absence of observed toxicity. Chemical analysis of the overlying water column in the tests indicated ammonia concentrations of 2 mg NH_4^+/L. These concentrations are lower than the reported NOEC of 4.68 mg/L total ammonia (ASTM Draft Annex-E724). Except for the sediment S1, all sediments resulted in acute effects (mortality) on the lugworm *Arenicola marina* (Results not shown). Ammonia toxicity may be responsible for the observed toxicity: The ammonia concentration in the pore water of S1 was almost 2 times lower than that of the other sediments (25 mg/L). However, prolongation of the test period to 21 days resulted in 100% mortality for all sediments, including S1. The lack of knowledge concerning the effects of long-term exposure in sub-optimal conditions (granulometric conditions, oxygen and food requirements, pH and salinity tolerance, sensitivity towards a number of 'natural' compounds like ammonia, nitrate, nitrite and chlorides) makes a scientifically-based interpretation of these toxicity data very difficult.

4. CONCLUSION

The development of an ecotoxicological assessment framework for marine sediments is still in its infancy: only few sediment toxicity tests have been described, standardized and validated. Several international organisations have advocated the use of a number of possible test procedures for the ecotoxicological evaluation of marine sediments, however, a specific, multi-trophic test battery has not been proposed yet. The "SETAC guidance document on sediment toxicity tests and bioassays for freshwater and marine environments" (1993) refers to 17 different species which can be used for marine sediment toxicity testing (amphipods, copepods, polychaetes, bivalves, decapods, fish, sea urchins). The JAMP-Guidelines (1998) and a report of the International Council for the Exploration of the Sea (1994) also give an overview of possible test species. Neither of these documents suggest a multi-trophic test battery for screening contaminated sediments. The latter was the main aim of the current study.

The algal growth inhibition assay with *P. tricornutum* was able to detect toxic effects which were not caused by the presence of elevated ammonia concentrations. This porewater test also did not detect toxicity in the reference sediments. However, during test performance colloidal particles appeared in the highest concentrations tested, possibly causing 'false positives'. Therefore, the algal test was not selected for the screening battery. No acute effects were detected with the rotifer B*. plicatilis* and with Microtox^R. Possible explanations for the absence of toxic signals are that 1) the tests are not sensitive enough or 2) the porewater of the examined sediments was not the most important exposure route for the contaminants found in these sediments. In view of their international acceptance, their cost-efficiency and the limited number of evaluated sediments, both tests are included in the final test battery for the time being. In order to enlarge the multi-trophic character of the battery, the acute test with the copepod *A. tonsa* is added to the battery. The occurrence of a natural contaminant such as ammonia, may cause serious problems for performing and interpreting the toxicity test results with a number of benthic organisms. In this context, the acute copepod assay is highly suitable for the detection of ammonia-related toxicity. Based on the results of this study the 10 d-whole sediment assay with the amphipod *C. volutator* is an acceptable candidate for the test battery. This mortality test shows sufficient discriminative power: although the sediment samples S3, S4 and S5 belonged to the so-called grey zone, this assay with *C. volutator* clearly identified the sediments potentially posing a risk to the environment. Indeed, no ecotoxicogical effect was found for S3. Significant effects were detected for S4 and S5 with the same assay.

The whole sediment bioassay with the lugworm *A. marina* seems to be a sensitive and ecological relevant test which can offer additional information on the ecological consequences of sediment and dredging spoils disposal. Incorporation of the lugworm assay in a test battery is, however, not advised since further research on standardisation an optimalisation of the assay is needed.

Based on the results of the present (limited) study the following test battery is proposed: porewater tests with *Acartia tonsa* (48h), *Vibrio fischeri* (15-min Microtox^{R}assay), *Brachionus plicatilis* (24h) and the 10 day bulk sediment assay with *Corophium volutator*. Further research on the use of the whole sediment assay with *A. marina* is recommended.

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Federal regulations for the disposal of dredged material in German coastal areas – experiences with chemical and biological criteria

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Introduction

For the management of dredged material in coastal and inland waterways under the jurisdiction of the Federal Waterways and Navigation Administration (*WSV*), two directives were developed. The regulations applicable to the aquatic disposal of dredged material in coastal waters (*HABAK-WSV*) are based on international guidelines provided by the International Conventions for the prevention of marine pollution (OSPAR-, HELSINKI- and LONDON-Conventions). Land disposal of dredged material from coastal waters and land as well as aquatic disposal of material from inland waterways are ruled by federal regulations for the management of dredged material in inland waterways (*HABAB-WSV*). The boundary between coastal and inland waterways is defined by the freshwater limit.

Disposal of dredged material from waters under the responsibility of the Federal States (*Bundesländer*) is governed by specific regulations. Currently, harmonisation of the different directives of the Federal States and the Federal Waterways and Navigation Administration is discussed.

The federal regulations include a sequence of activities and the scope of investigations provided for by the international guidelines. Investigations comprise the physical description, chemical analyses and ecotoxicological tests, both of sediments at the dredging site and at the disposal site. Furthermore, biological investigations, e.g. of benthic communities at the disposal site, are required. According to the international guidelines, dredged material composed mainly of sand, gravel, rock or previously undisturbed geological material may be exempted from chemical analyses and biological testing. The decision on beneficial use or disposal options is based on the results of the investigations. In the case of aquatic disposal, a monitoring programme is designed for the disposal area and the predicted zone of impact.

Chemical investigations

Generally, information is required on trace metals and arsenic, di- and tributyl tin compounds, total hydrocarbons, PAHs, CBs and a number of further organochlorine compounds, that still are widespread in German coastal waterways. If local sources of contamination or historic inputs are suspected, analyses of additional determinands may be necessary. For fine sediments, oxygen and nutrient budgets have to be assessed additionally .

Following the recommendations of the OSPAR Dredged Material Guidelines, lower and upper guide values were defined for distinguishing three cases. The guide values represent management values and are neither ecotoxicological quality criteria nor quality targets. They were derived from the contamination of sediments from the 0Wadden Sea, an area receiving disposed dredged material to some extent, and they refer to the fine fraction < 20 µm. Guide values for organotin compounds are still under discussion. For oxygen depletion, too, no guide values exist, however, the oxygen depletion resulting from the disposal of dredged material has to be estimated.

Currently, these guide values are only applicable to dredged material from the federal waterways. In addition to the guide values, the actual regional contamination and other regional characteristics of the disposal area should be taken into account when assessing contamination of dredged material.

Ecotoxicological testing

Chemical analyses cannot cover all contaminants that may be present in dredged material in toxicologically relevant concentrations. Therefore, bioassays are performed, whenever an ecotoxicological risk cannot be excluded. The OSPAR Dredged Material Guidelines recommend to use a set of two to four bioassays with different taxonomic groups. However, currently routine testing is restricted to the luminescent bacteria test with *Vibrio fischeri* in pore water and eluates, as this is the only marine toxicity test standardised in Germany by now. In case of elevated pollution level, and a negative response in the luminescent bacteria test, the use of other, however not yet standardised tests is considered. The standardisation of further tests for marine application is being prepared in Germany. A battery of three standardised tests is intended for routine testing of marine samples, as already practised for freshwater sediments.

In order to describe the ecotoxicological potential of dredged material, an evaluation method was developed in the Federal Institute of Hydrology, Koblenz. Tests are made with pore water, eluates or extracts, that are diluted in geometric sequence with a dilution factor of two. Toxicity is quantified by the first dilution step showing no toxic effects and is expressed as the "pT-value", i.e. the negative binary logarithm of this dilution step. The pT-value indicates, how many times a sample must be diluted in the ratio 1:2 in order to show no more toxic effect. The scientifically based classification system consists of seven classes. The most sensitive test determines the toxicity class. For management purposes, toxicity classes are assigned to 3 cases

Biological investigations at the disposal site

Biological surveys are required in order to assess the potential impacts on biological communities in the disposal area, e.g. by smothering of benthic organisms, increased turbidity or modification of sediment composition. Investigations include composition and distribution of macrozoobenthos and fish, as well as special habitats or endangered species.

Decision on disposal options

As mentioned above, with regard to the management of dredged material, 3 cases are distinguished according to the results of contaminant and nutrient analyses, as well as of ecotoxicological tests. The criterion classified worst determines the total classification of the dredged material.

Dredged material classified as case 1, can be disposed of in the coastal area, taking into account only physical and biological effects. If the material is categorised as case 2, aquatic disposal is regarded as acceptable, provided an impact assessment shows that disposal areas and legal uses are not affected significantly. In the long run, no significant accumulation of contaminants should occur, and toxicity of sediments should not increase due to disposal. Dredged material of case 3 requires, additionally to considerations under case 1 and 2, an impact assessment including a comprehensive comparative assessment of aquatic and land disposal options. If land disposal is found to be more acceptable, aquatic disposal should not be permitted. However, dredged material assigned to case 3 is not automatically excluded from aquatic disposal.

The impact assessment is derived from the results of chemical, ecotoxicological and biological investigations, taking into account further information on, e.g., morphology, hydrodynamic conditions and potential uses of the disposal area. Based on the impact hypothesis, the best disposal option is selected, and if applicable, a monitoring programme at the disposal site is designed.

Application of federal regulations in coastal waterways: case studies

Maintenance dredging in the entrance basin connecting the Elbe to the Nord-Ostsee-Kanal, Brunsbüttel:

The dredging site is situated in the turbidity zone of the Elbe estuary. Due to high concentrations of suspended particulate matter and high sedimentation rates, 5 – 9 million m^3 sediments / year have to be dredged to maintain the navigable depth. The material is disposed of a few kilometres downstream the dredging site in the Elbe.

With regard to trace metal and nutrient concentrations, this dredged material was assigned to case 2. Concentrations of a few organic contaminants gave rise to classification in case 3. However, the impact assessment gave no indication of additional contaminant input into the water body due to dredged material disposal: contaminants analysed in the dredged material were in the same concentration range as in suspended particulate matter near the dredging site as well as in sediments in the disposal area. Dredged material mainly consists of suspended particulate matter of the Elbe that already shows elevated contaminant concentrations and settles fast. Due to hydrodynamic conditions, disposed dredged material is even transported back to the dredging site to some extent, and therefore, the same material with its associated contaminants has to be dredged repeatedly.

Ecotoxicological testing was carried out with 6 different tests, including sediment, pore water and eluate tests. The highest toxicity with a pT-value of 2 was observed with the green algae reproduction test (*Scenedesmus suspicatus*) using eluates for samples from the dredging site; samples from the disposal site showed a pT-value of 1. The *Daphnia magna* test in pore water showed a pT-value of 1, both for a sample from the dredging and disposal site, however, no toxicity was observed using eluates. A plant test (*Lepidum sativum*) taking wet weight as the measure and a hydroid reproduction test (*Cordylophora caspia*), both performed with eluates, showed a pT-value of 1 for dredged material samples. The only test with sediment, carried out with *Corophium volutator*, did not show any toxicity. With regard to ecotoxicological testing, all sediments tested belong to case 1, i.e. the dredged material as well as the sediments at the disposal site are regarded as non-toxic to slightly toxic.

Results of the ecotoxicological tests did not correlate strictly to contaminant concentrations, maybe since only small differences were observed, both, for toxicity and contaminant concentrations between samples.

Due to natural conditions in the turbiditiy zone of the river Elbe, macrozoobenthic communities generally show reduced species abundance. A further significant decrease of the number of species was observed at the disposal site compared with reference areas. The impact, resulting from continuous disposal of large quantities of dredged material, is restricted to the disposal site and its direct surroundings. However, for fish population and diversity, and fisheries, no impacts were expected.

Although an impact on macrozoobenthic communities was detected, aquatic disposal is selected as option of least detriment, as this impact is restricted to a small area and is therefore regarded as more acceptable than the use of large areas for disposal of 5 – 9 million m^3 dredged material / year on land. No impact is expected to result from contaminants and ecotoxicity.

Maintenance dredging in the Weser estuary, Bremerhaven:

In the Weser estuary, only ca 600 000 m^3 material / year have to be dredged. The material dredged was classified as case 2. The disposal of the small amounts of dredged material did not show a significant impact on macrozoobenthos and fish. Only at disposal sites for silty material were the number of species and their abundancies slightly reduced. It was decided that aquatic disposal can be continued without restrictions.

Currently, several further investigations according to the federal regulations for the management of dredged material in coastal areas are being carried out or are planned.

Future requirements

The ′Proposed List of Priority Substances in the Context of the EU Water Framework Directive′ includes several compounds that are relevant for sediments but are not yet covered by the routine analytical requirements for dredged material, e.g. brominated diphenylethers or octyl- and nonyl phenols. Further biotests addressed by the OSPAR Dredged Material Guidelines include microcosm and mesocosm experiments for short and long-term effects as well as biomarkers. According to theses proposals, in future, further requirements with regard to chemical and biological testing possibly should be considered in regulations for the management of dredged material.

Strategies for the assessment of sediment quality
- Identification of culprit chemicals -

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Introduction

The European Water Framework Directive demands to establish river basin management plans in order to provide a sound basis for measures to be undertaken to meet quality criteria for surface waters, ground water, and coastal waters.

In western Europe in the past years environmental protection measures have led to a reduction of the contamination of surface waters as well as to a shift form the impact of point-emissions to diffuse sources. However still quality criteria for a number of compounds (e.g. PAH, PCB) are often exceeded.

A major issue from the ecological as well as from the economic point of view are contaminated sediments / dredged material. Generally there is a gap of knowledge about the linkage of contaminants in dredged material to their upstream emission sources. This might be one reason why the polluters-pay-principle is not yet implemented. One of the tasks to be undertaken as a prerequisite is the identification of culprit chemicals.

Currently quality criteria and management guidelines are defined on national as well as on international levels (e.g. OSPAR dredging guideline [1]).

It is expected that future policies for the assessment of dredged material will implement bioassays as addition to the chemical criteria [2,3]. In addition the present list of chemical criteria is limited and new compounds, e.g. OSPAR 1998 list of candidate substances [4], [5], are merging as likely candidates for inclusion in regulatory frameworks.

Strategies for the assessment of sediment quality

The European Inventory of Existing Chemical Substances lists over 100 000 commercial chemicals. The threat posed by many of these compounds remains uncertain because of the lack of knowledge about their concentrations and the ways in which they are transported, transformed, and accumulated and then impact on humans and wildlife.

Present monitoring concepts can be divided in two main categories with characteristic advantages (+) as well as disadvantages (-):

Ø Chemical-analytical methods

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

Ø Ecotoxicological methods

 + integrated assessment of effects or toxic potentials

 - 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.

There is some consensus that for the sake of being cost-effective and as well to outrule the described disadvantages a three step (multi-level) approach for monitoring should be followed [6]:

1. Trend monitoring only for a restricted number of single chemical compounds and physical base parameters.
2. Screening with biological methods (bioassays, biomarkers, biosensors) to identify regional areas of concern.
3. In-depth-investigations to evaluate the key parameters responsible for observed effects or potential hazards (combining chemical analysis and detailed biological studies) in the areas of concern.

Ad 1. According to current and future policies on dredged sediments.

Ad 2. It is expected that future policies for the assessment of disposable dredged materials will include bioassays (e.g. test-systems for acute toxicity as well as benthic organisms of different trophic levels) [2 ,3]. In the scientific community there is some consensus that only a battery of tests incorporating different toxicological endpoints as well as organisms from different trophic levels will provide adequate results [7].

Ad 3. Different strategies combining chemical-analytical and biological/toxicological methods have been developed to identify chemicals which mainly contribute to the observed effects or toxic potentials, e.g. US-EPA Toxicity Identification Evaluation (TIE protocols [8]) or Bioassay-Directed Chemical Analysis (BDCA, Fig.1, below) and have been applied successfully to the investigation of contaminated sediments [9-11].

Identification of culprit chemicals – Bioassay-directed chemical analysis

The bioassay-directed chemical analysis approach

Chemical-analytical methods and bioassays are combined in an approach called Bioassay-Directed Chemical Analysis (BDCA) which is outlined in Fig.1. In this iterative process of extraction, fractionation and the application of bioassays, the chemical non-target screening of potentially toxic fractions leads to the identification of compounds which contribute significantly to the overall toxicity of the sample.

Ø Matrices: e.g. sediment, water

Ø Various extraction methods, e.g. liquid/liquid and liquid/solid extraction

Ø Fractionation techniques: various column chromatographical procedures, reverse and normal phase HPLC, GPC

Ø Effect screening: bioluminescence assays for acute toxicity and mutagenicity (Microtox^{TM}, Mutatox^{TM}), yeast screen assay for endocrine disrupting chemicals. More sophisticated bioassays (e.g. DNA-damage, EROD, fish embryo development) have been tested in on-going co-operations with other institutes.

- Ø Chemical non-target screening: GC/MS, HPLC/MS (ESI, APCI, particle beam)
- Ø Chemical target analysis: GC/ECD, GC/NPD (TID), GC/MS, GC/MS/MS, HPLC/MS/MS

BDCA studies on sediments

Sediment samples of the Elbe catchment area including Hamburg Harbour as well as other sites, e.g. Hamilton Harbour, Canada, were investigated with BDCA utilising different bioassays [9-11].

The example given in Fig. 2 highlights the identification of man-made chemicals contributing to the acute toxic potential of a river sediment but also reveals that natural compounds as elemental sulphur or cholesterol derivates can not be neglected.

Extracts of this sediment sample from the Bilina river - a tributary of the Elbe in the Czech Republic - showed high acute toxic potentials. Bisphenol A (BPA), known for its esterogenic effects, was identified to contribute among other compounds to the acute toxic potential of the sample. In the sequentially derived extracts more than 100 compounds were identified. Fig. 2 depicts the effect screening and chemical non-target analysis after fractionation of one of the extracts (methanol, pH7) of the Bilina sediment sample.

The acute toxic potential linked to BPA was confirmed by synthetic samples (lab water spiked with quantified amount of BPA in sediment extract).

Bisphenol A (BPA), known for its estrogenic effects, was observed in high concentrations in sediment samples from the Bilina river. It was obviously stemming from a chemical plant producing epoxy resins. Due to its low octanol-water partitioning coefficient (log Kow = 2.2-3.8) it was expected to be mainly transported in the water phase downstream the Elbe river.

Regional hot spot or hazard to the aquatic ecosystem of the Elbe river?

In a follow-up study quantitative analyses of BPA in a length profile of the Elbe river and additional investigations revealed that the high BPA concentrations (above 1 µg/L) in the Bilina rapidly decreased downstream in the Elbe river, obviously due to dilution as well as degradation.

With regard to the relative estrogenic potency of BPA (10^{-3} - 10^{-4} compared to 17-ß-estradiol and DES) the observed concentrations downstream in the middle and lower Elbe (50 ng/l or lower) were not suspected to be hazardous to the aquatic ecosystem although in terms of mixture toxicity they contribute to the estrogenic potential in the Elbe river.

Conclusions

- Ø Bioassay-directed chemical analysis leads to the identification of toxic compounds not yet covered by present monitoring programs.
- Ø It is a tool for cost effective case studies in terms of pre-selection of sampling sites and narrowing the broad spectrum of relevant chemicals for quantitative analysis.
- Ø The results of these shortly described studies therefore enable to focus on the relevant chemicals and provide together with studies on emissions and mass flows the basis for

action plains to reduce the so-identified priority pollutants in order to fulfill on-coming guidelines for the disposal of dredged materials.

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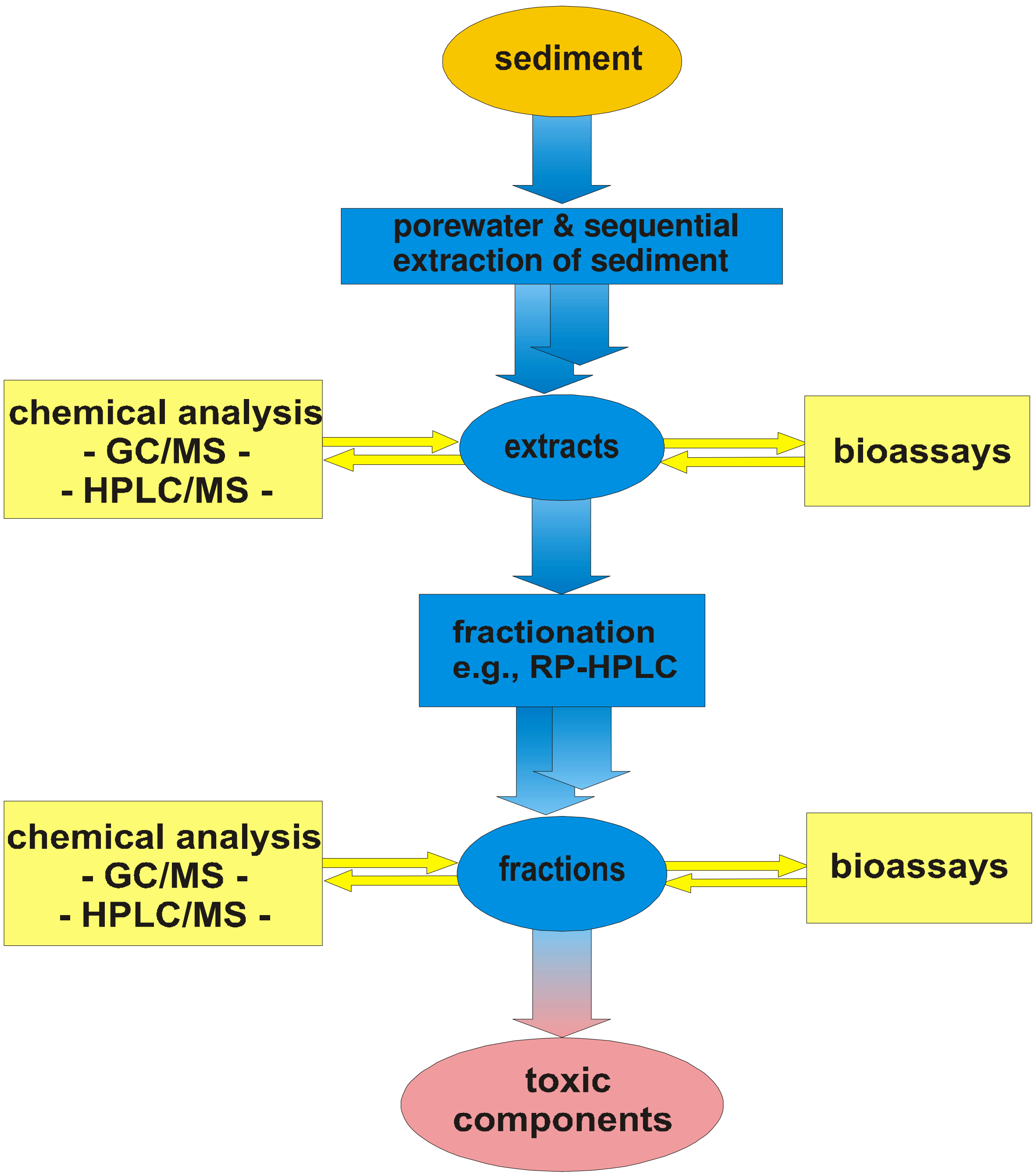

Fig. 1: Experimental concept of Bioassay-Directed Chemical Analysis

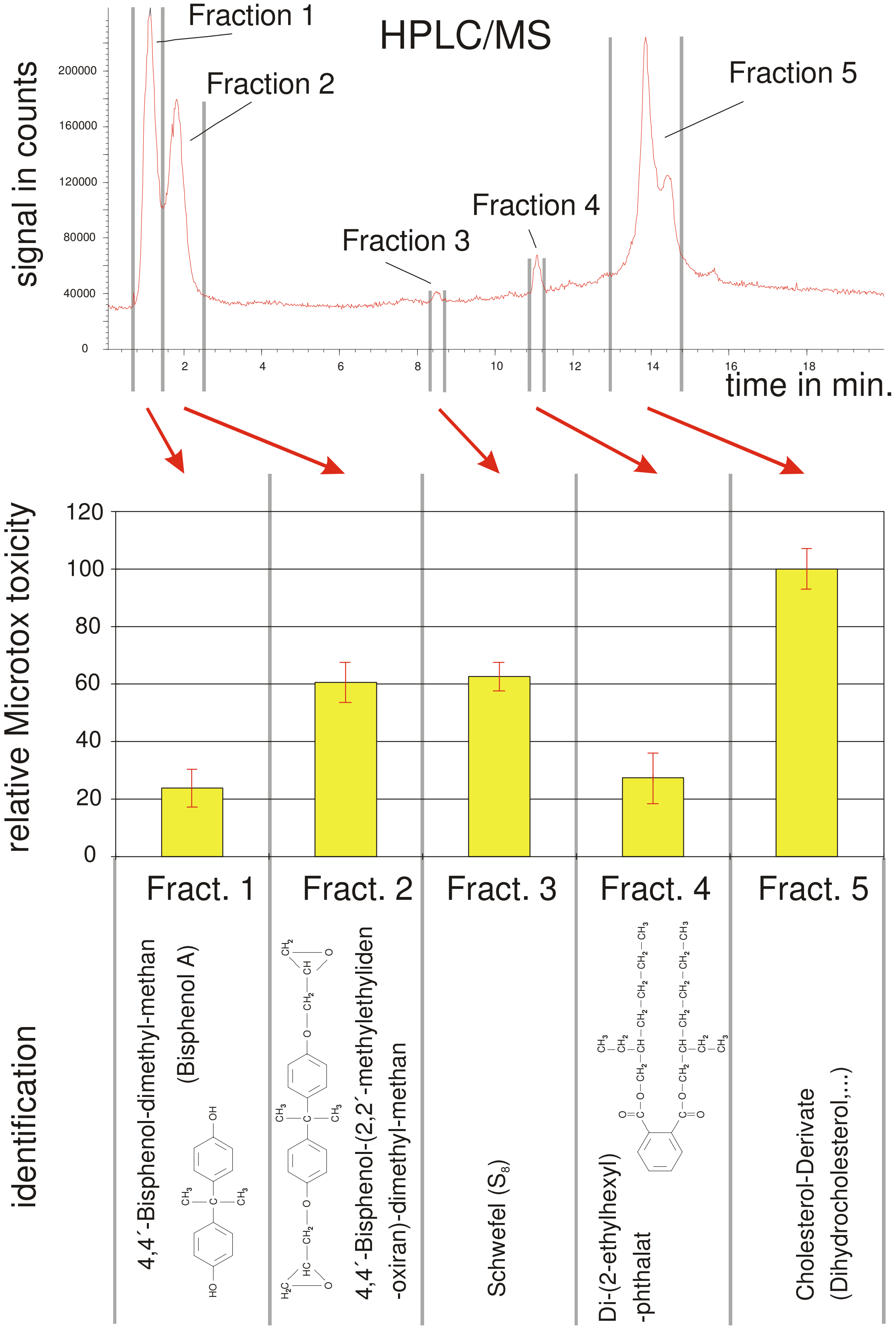

Fig. 2: Example for the identification of anthropogenic as well as natural compounds contributing to acute toxic potential

The problematic of contaminated sediments: A general view of the situation in Spain

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A Large number of European estuaries and rivers is contaminated with a range of pollutants as a result of inputs of materials over decades to centuries. Probably the most persistent and potentially most harmful contaminants in the system are heavy metals and certain organic micro-pollutants (e.g. PCBs, PAHs). Improved understanding of estuarine/riverine sediments as reservoir of key organic and inorganic pollutants, and exchange between this reservoir and water column and via food chains to man, are therefore of major European interest as regards improvement of quality of the environment and enhancing the national assets which estuaries represent.

The majority of coastal regions in Spain and in Europe are serviced by shipping that requires navigable channels to operate and supply the local industries, and for the transfer of goods. Most of these channels gradually infill, and thus dredging of these often polluted sediments is inevitable, with potential remobilisation of pollutants and consequent risks to environmental quality and public health. It is only recently that such risks have been considered, but as little is known about the real environmental consequences of dredging activities, making rational management decisions has been difficult.

However, despite the potential environmental risks associated with the removal of contaminated sediments, substantial amounts of dredged sediments are dumped into the sea or used as landfill for shoreline modification, wetland restoration, sanitary cover and agricultural soil replenishment. The scale of these problems both financially and in terms of masses of material can be very great (e.g. the Slufter project in the Netherlands; Nijssen et al., 1997) and significant effort has gone into trying to remediate such contaminated materials (e.g. Detzner et al., 1997). In the Spanish harbours, the average annual volume of material dredged during the period of 1988-1998 was 7.850.000 m^3, and 3.250.000 m^3 was the average annual volume of material disposed into the sea. This quantity represents the 46 % of the removed material, which is a considerable amount of sediments dumped into the sea every year with a potential environmental-economical-social and political impact that needs to be considered.

The increasing concern in recent decades over the environmental impact of contaminated sediments is reflected in stricter regulations implemented at national level by, for example, USA organizations. At this national level, legislation varies greatly, but there are three basic approaches i.e. use of standards (based on total contaminant load), toxicological effects on organisms, or using a “case by case” strategy.

In Spain, the document ***"Recommendations for the Management of Dredged Material in the Ports of Spain"*** (CEDEX, 1994) elaborated in 1994 by representatives of diverse Institutions as Port Authority, General Directorate for the Merchant Marine, General Secretariat for Marine Fishery, Spanish Oceanographic Institute, General Directorate for Environmental Policy, General directorate for Coasts, the Minister of Public Works, is the guide to classify the dredged material in three different categories established as a function of the chemical and/or biological nature and the effects they may produce on the marine biota. These three categories are defined as follows:

Category I: belong to this category those materials which come from the dredging of the port bottoms whose chemical and/or biological effects on marine flora and fauna are null or practically insignificant. The materials dredged belonging to this category can be freely dumped into the sea with the sole consideration of the mechanical effects.

Category II: belong to this category those dredged materials with moderate concentrations of contaminants. These can be dumped into the sea in a controlled manner (prior selection of the dumping site; the formulation of an impact hypothesis which predicts the physical, chemical and biological effects on the marine environment will be necessary; an environment surveillance programme will be follow to insure that the levels of environmental impact do not surpass those evaluated in the impact hypothesis).

Category III: belong to this category those dredged materials with elevated concentrations of contaminants. These materials must be isolated from sea waters or subjected to adequate treatment to impede or minimise the bioavailability of contaminants. Two subcategories are distinguished as a function of the degree of contamination of the sediments:

- Sub-category III a): soft isolation techniques for the management of dredged materials can be employed.
- Sub-category III b): hard isolation or treatment techniques for the management of dredged materials must be employed.

Prior to the above classification, the characterisation of the sediments to be dredged must be done. These studies are based on granulometric analysis, chemical analysis of total organic carbon (TOC), heavy metals (Hg, Cd, Pb, Cu, Zn, Ni, Cr) and organic compounds (PCBs). The biological studies include the estimation of the toxic effect in the short term, the estimation of the toxic effect in the long term to determine sublethal effects, and the estimation of the biological assimilation of harmful substances.

The Department of Oceanography and Marine Environment of AZTI is involved in a surveillance programme on estuarine and coastal waters quality in the Basque Country since 1994. This programme comprise the studies of water, sediments and biota to determinate the spatial distribution of contaminants and their origin, and to evaluate the contaminants levels. An intensive study is has been done in six estuaries: Nervión, Barbadún, Lea, Oka, Butrón and Artibai. A total of 183 sediment surface samples have been analysed in terms of heavy metals and organic compounds (PCBs, PAHs, DDTs, HCB and HCHs.

Since the problem generated with contaminated sediments, the need of dredging and disposal is a global problem for developed and developing nations (see e.g. USA Environmental Protection Agency management strategy for contaminated sediments) is inferred the necessity to unify criteria an guidelines for sediment quality assessment. An efforts for collaboration with other countries in Europe have been made in AZTI through an European proposal, which intents to provide a scientific basis of the consequences of dredging. As regards transport and fate of these pollutants and impact on ecosystems is the focus of this proposal, which has the intention of providing a stronger scientific framework for formulation of policy and practical directives on dredging and dumping of contaminated sediments. The research programme will be carried out in the estuary of Bilbao which is one of the most contaminated areas in Spain.

The prime scientific motivation of this proposal is thus to quantify the transport of contaminants (heavy metals and organic contaminants) from sediments to the water column (dredging activities and benthic fluxes), the fate of sediment-bound contaminants and their transport pathways, impact of pollutants on biological communities, and to study remediation strategies, and production of decision support systems for end-users. Overall the proposed work will therefore study the impact of anthropogenic activities in these near-shore marine systems, and make use of the knowledge to contribute to improving the quality and sustainability of these systems containing contaminated sediments, in order to improve water quality and environmental conditions. Thus findings will aim to inform policy-making, and help establish Ecological Quality Objectives, and in the development of Community Environmental legislation.

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The necessity of a marine bioassay test-set to assess marine sediment quality and its approach

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Necessity

Because of the rather limited understanding of the chemical bioavailability it is necessary to use bioassays, i.e. suborganism-, monospecies- and multispecies ecotoxicity test. Therefore bioassays are necessary for the assessment of marine sediment quality (immission analysis) and subsequently should lead to the formulation of emission standards of single substances and substance mixtures, e.g. dredged material.

From the legal point of view marine bioassays for the assessment of sediment quality and dredged material are required because of the obligations arising from the international regulations for the protection of the North Sea and the Baltic Sea (OSPAR and Helsinki conventions). In the German environmental law there still is a lack of marine bioassays assessing the impact of compounds to marine sediment quality.

Approach

Several marine bioassays have been developed, which are standardized to different degrees. As the test organisms vary in their sensitivity to physico-chemical compounds and as there is no most sensitive species found yet, and probably never will, it is necessary to combine several bioassays as a test-set („battery").

In the frame of the project „Validieren, Harmonisieren und Implementieren eines minimalen biologischen Testsets zur Bewertung mariner Wasser- und Sedimentproben" (funded by the German Environmental Protection Agency, started in November 1999) and in cooperation with the DIN working group „Marine Biotests" the following criteria for the bioassay - selection for the minimal test-set, have been applied:

- The bioassay has to be a monospecies test.
- The test-set should be sensitive to a wide range of toxicants.
- The selected species should represent different trophic levels.
- The test-set should cover different exposure routes:
    - Via the waterphase (porewater and sediment overlying water) and
    - directly from solid-bound contaminants).
- The test species should be local, i.e. German, species.
- Tests should be standardized (e.g. as an ISO-Guideline).
- Tests should be reasonable practicable.

- The test costs should be adequate to the results delivered.

Considering these criteria the following bioassays were chosen for the test-set (Tab. 1):

Tab. 1: Bioassay test-set to assess sediment quality.

Test	Test species	Parameter	Duration
Luminescent Bacteria Acute Toxicity Test	*Photobacterium phosphoreum* (Syn. *Vibrio fisheri*)	Reduction of light emission	30 minutes
Marine Algae Toxicity Test	*Phaeodactylum tricornutum* or *Skeletonema costatum*	Growth (reproduction) inhibition	72 hours
Amphipod Acute Sediment Toxicity Test	*Corophium volutator*	Mortality (survival), burial	10 days

The major tasks of the project are:

- Validation: Is this test-set suitable to assess the quality of sediment, porewater and elutriates ?
- Harmonization: The methods used in the different bioassays have to be modified to grant best possible congruence and identical treatment of the samples.
- Implementation: The test-set should be established by a sufficient number of applicants by round robin tests during a period of two years.

3 Posters presented

1. "Rhine Research Project for a sustainable port"; Rotterdam Municipal Port Management, Rotterdam, The Netherlands.
2. "Rhine Research Project II", Rotterdam Municipal Port Management, Rotterdam, The Netherlands.
3. "Selection of a test battery for the ecotoxicological evaluation of marine sediments"; D. G. Heijerick, M. Vangheluwe, C. R. Janssen, G. Dumon; Ghent University; Laboratory for Environmental Toxicology and Aquatic Ecology, Gent, Belgium.
4. "Ammonia toxicity in marine harbour sediments in the Netherlands"; M. E. Schot, M.C. Dubbeldam, J. Stronkhorst, Ministry of Transport, Public Works and Water Management; National Institute for Coastal and Marine Management/ RIKZ, Den Haag, The Netherlands.
5. "Quality assurance for five sediment toxicity tests"; C. Schipper, J. Stronkhorst, Ministry of Transport, Public Works and Water Management; National Institute for Coastal and Marine Management/ RIKZ, Den Haag, The Netherlands.
6. "Bioassay-Directed Chemical Analysis - A case study of Bisphenol A in the Elber river"; J. Gandrass, J. Kurz, B. Schmidt, A. Zimmermann, GKSS Research Centre, Institute of Physical and Chemical Analysis, Geesthacht, Germany.
7. "Identification of toxic relevant organic chemicals in sediments using bioassay-directed chemical analysis“; J. Kurz, GKSS Research Centre, Institute of Physical and Chemical Analysis, Geesthacht, Germany.
8. "What causes toxicity in your effluent, surface water, sludge or soil?"; J. Brils, TNO-MEP, Den Helder, The Netherlands.
9. "The pT-Value - An Ecotoxicological Classification Index for Sediments and Dredged Material“. F. Krebs, Federal Institute of Hydrology, Koblenz, Berlin, Germany.
10. "Ecotoxicity of various oil types in marine sediments and the effect of ageing"; M. Scholten, J. Lourens, T. Crommentuijn, S. Huwer; TNO Institue of Environmental Sciences, Energy Research and Process Innovation, Dept. for Ecotoxicological Risk Studies, Den Helder ; National Institute for Coastal and Marine Management / RIKZ; National Institute of Public Health and the Environment, RIVM, The Netherlands.

4 Report of Working Group I (science-oriented)

Chair: Bram Brouwer, Rapporteur: Susanne Heise

Jos M. Brils, Marc Eisma, Lee Grapentine, Carolin Peters, Cor Schipper, Maria J. Belzunce Segarra, Marnix Vangheluwe

The discussion in the working group focussed on 3 subjects:

1) the strategy of biotests, 2) the applicability of the Toxicity Identification Evaluation-Method (TIE) and 3) the use of biotests in an Ecological Risk Assessment (ERA).

The participants agreed that the ultimate goal of biotest application should be the assessment of possible risks for the receiving environment. Momentarily, however, it's use is limited to the identification of hazards of dredged materials and therewith to the description of the present status rather than a prediction of possible effects.

1) Strategy of biotesting

Criteria for biotests should be ecologically relevant and thus consider different exposure routes and feeding regimes. Therefore whole sediment tests with sediment-dwelling organisms of different feeding habits should be preferred: These organisms cover pore-water exposures as well as direct exposure due to different kinds of ingestion or bodywall contact.

However, if a stringent correlation between pore water and whole sediment test results is evident, pore water testing as a supplementary test system can be used for practical reasons.

Regarding their general applicability for routine measurements, those tests should be preferred, that are easily repeatable, reproducible and to a minimal extent be biased by confounding factors. They should be standardised and validated, so that procedures are well worked out and documented, and differences between laboratories can be kept low.

Concerning the interpretation of biotest results: those tests should be chosen that have a higher resolution, e.g. by showing a wide range of dosis-effect responses rather than binary (yes or no) results, and therewith enable a gradual quantification of toxicity.

Toxicity results in the first tier should give information about general toxicity of the sediment and thus demand that no toxic effects are missed. Biotests that are applied during this first stage should thus be sensitive to a broad spectrum of chemicals and include different effect-modes.

Although a lot of those substances can be detected by acute toxicity tests, especially mutagenic, developmental and endocrine effects will only show up in chronic test systems which are currently not well developed and established for marine sediments. An urgent need for research in this direction has been identified during this workshop.

To prevent the need for extensive use of chronic, long-term, testing for sediment toxicity evaluation, the development and introduction of chemical-class specific biotests such as CALUX as a quick discriminator of endocrine and developmental potency is strongly recommended.

As no single test will be able to fulfill all of the criteria stated, the application of a biotest battery is suggested which might also include different trophic levels. It should be composed in such a way, that it covers most chemicals, the most important effect-modi and the most significant exposure routes in order to minimise the number of false negative results, pretending wrongly, that no toxicity exists.

To exclude tests, giving redundant information, from a battery is preferred because of economical and effectivity reasons, but it is acknowledged, that a weight-of-evidence-approach can be useful where confidence in single tests is limited or when authorities have to be persuaded of extensive implications.

2) Toxicity Identification Evaluation (TIE)

The TIE approach is regarded as very promising to identify true causes of toxicity and should be used as a second tier to characterise the chemicals of concern in e.g. a sediment catchment area.

Attributing ecotoxicity in biotests to chemical compounds in a cause-effect-way is important for a refined risk assessment and for establishing emission reduction measures. A toxic signal in one of the TIE-steps could trigger and direct the performance of ecological risk assessments before a sediment can be disposed of. Additionally, by this method, new chemical compounds of concern may be identified whereby others might become regarded as being of minor ecological importance.

Mesocosm studies in connection with TIE could be used as an important scientific tool to investigate processes and pathways that connect biotest information and ecologically significant effects, hence giving information about bioavailabilities and exposures.

For relevance, these studies should be done on whole sediments instead of pore water, which has mostly been used in the past.

Research in the field of TIE development and application, however, is fragmented and largely uncoordinated. For effectivity reasons the formation of a thematic network for TIE research and development on the European level is strongly recommended.

3) Ecological Risk Assessment (ERA)

ERA has two elements: the probability of something happening and the consequences when it does. So it consists of the assessment of the status quo, which might involve the identification of a hazard, and the estimation of exposure scenarios and, following consequently, possible effects on organisms.

As exposure and effects can't be depicted from chemical analyses, biotests have to form a substantial element of ERA. By characterising substances of concern, modes of exposure and the sensitivity of organisms, e.g. with the help of mesocosm studies or on the basis of field bioassays, prediction of field impacts might become possible.

However the natural variability of toxicity in the receiving environment complicates the identification of add-on toxic effects by dredged material and, thus, must initially be quantified in order to differentiate baseline "natural" effects from add-on chemical-based effects in biotest results. Furthermore, different biotest responses can be due to variations between organisms, different samples of the sampling site and the confounding factors. Consideration of among-site variations therewith is of high importance.

However, the information of bioassays is not yet evaluated in terms of going from hazard to risk assessment.

Therefore it is strongly recommended to perform a full-scale field evaluation study (biotests, chemical analyses, ecological impacts) to confirm bioassay-based predictions of adverse effects of sediment disposal. This would represent an important first step in the implementation of biotests in Ecological Risk Analysis.

A proposal for such a study (BIOSAFE, (**Bio**logically Based **S**ediment Quality **A**ssessment by **F**ull Scale Field **E**valuation) was discussed and presented in the podium discussion of the workshop.

5 Report of Working Group II (application-oriented)

Chair: Remi Laane, Rapporteur: Juergen Gandrass

Ulrich Foerstner, Charlotte Hagner, Falk Krebs, Guilherme Lotufo, Peter Mollema, Axel Netzband, Birgit Schubert, Joost Stronkhorst, John Thain

Introduction – general statements

The discussion was driven by **scientific arguments** but it was tried to keep in mind that the ‘end users’, e.g. harbour authorities and regulatory authorities, have the stringent need **for reliable criteria that are easy to handle** (suitability for the decision-making processes involved in the management of dredged material).

However we intended to integrate **future perspectives** for the development of tools and strategies. From a practical point of view these would have to be run in a first stage including standardisation and harmonisation in a ‘research mode’ to prove their applicability and then in the second stage could be implemented into guidelines / regulations.

In the working group there was consensus that the discussion on bioassays as additional criteria for the assessment of the quality of dredged material has to take into account the feasibility of implementation into a **decision-making framework for the management of dredged material.**

This framework has not only to cover hazard assessment at the dredging site and if possible risk assessment with regard to the relocation of dredged material but has also to take different management options with all their pros and cons, their feasibility etc. into account.

It has been recognised that, to a large extent, contamination of dredged material is due to the prevailing concentrations of contaminants in suspended particulate matter that settles in areas of low energy and has to be dredged and relocated in order to maintain navigation.

In that regard the following general demands were recognised:

With regard to policies and regulations **a responsible dredged material management** is needed but at the same time the **main aim should be to reduce emissions from point sources as well from diffuse sources in the catchment area**.

The latter would ensure in the long-term

(1) the **reduction of inputs from rivers directly into the marine environment** via suspended particulate matter (SPM) carrying contaminants,

(2) to **enable the relocation of dredged material** in the rivers itself as well as in the marine environment,

(3) to **enable different sorts of beneficial uses** (nourishment, mudflats, habitat-creation),

(4) to stop the need for expensive land-based treatment and/or safe disposal of contaminated dredged material,

without imposing unacceptable threats to the aquatic ecosystem.

In the following a summary of conclusions and recommendations is given on questions like the availability of suitable bioassays, approaches towards decision making processes, methods to assess sediment toxicity data and how to translate data from laboratory experiments to field situations.

Question 1: *Are presently suitable bioassays or batteries of tests available?*

With regard to decisions on the disposal of dredged material in the marine environment available bioassays are listed (Table 1) that are already standardised, i.e. agreed methods are available with standard operating procedures (SOPs) and quality assurance / control measures (QA/C). As well the endpoints, the used matrices (whole sediment, aqueous phase, extract) and their current use (dredged material, notification[1], monitoring, research) are stated.

Conclusions

- Ø A set of a few **standardised bioassays** for testing whole sediments as well as aqueous phases (pore-waters, elutriates, extracts) **are available** for testing dredged materials as well as for other purposes.
- Ø The endpoints of these bioassays can mainly be summarised as looking at the **acute toxic** potential.
- Ø Some of them, e.g. Microtox®, *Corophium volutator*, have already been harmonised on a European level by intercalibration (e.g. Round-Robin Tests).
- Ø Harmonisation could depend on the regional availability of the species.
- Ø A shift from tests for acute toxicity towards chronic toxicity is expected.
- Ø There is a **developing field of chronic tests and receptor-based tests / biomarkers** which are mostly not yet standardised. As example the DER CALUX assay for chemicals with dioxin-like modes of action is included in Table 1.

Recommendations

- Ø A **battery of tests with 3-4 bioassays** listed in Table 1 including **at least one whole sediment test** should be carried out.
- Ø The **development of chronic tests and receptor-based assays /biomarkers** should be carried out. Standardisation and harmonisation is necessary.
  Arguments are: (1) chronic tests could be more sensitive and cover as well other modes of action of chemicals than tests for acute toxicity. (2) receptor-based assays / biomarkers may cover persistent bioaccumulating compounds otherwise overlooked and might in future replace chemical analysis undertaken at high costs, e.g. CALUX assay for chemicals with dioxin-like mode of action.

[1] Requirements of testing for the registration of ‚new chemicals‘

Table 1: Available standardised bioassays for the assessment of dredged material and for other purposes

bioassay	**endpoint**	agreed method **SOP**	agreed method **QA/C**	**matrix**	in use for **dredged material**	in use for **notification**	in use for **monitoring**	in use for **research**
Amphipods (e.g. *Corophium volutator*)	mortality	X	X	whole sediment	X	X	X	X
	chronic	X	X	whole sediment	-	-	X	X
Algae	growth inhib.	X	X	aqueous phase	-	X	-	X
Polychaete (e.g. *A. marina*)	mortality, grothw, casting	X	X	whole sediment	X	-	X	X
Sea urchins (e.g. *E. cordatum*)	mortality, reburrowing	X	X	whole sediment	X	-	X	-
	development	X	X	aqueous phase	-	-	X	X
Bacteria (e.g. Microtox®)	bio-luminescence	X	X	aqueous phase, suspended sediment	X	?	X	X
Mollusc larvae (e.g. oyster larvae)	development	X	X	aqueous phase	X	-	X	X
Copepod (e.g. Tisbe, *A. tonsa*)	survival, reproduction	X	X	aqueous phase	X	X	X	X
Cell-lines (e.g. DRE CALUX)	dioxin like mode of action	X	-	extract	X	-	X	X

- Ø The bioassays should not only be **applied** to dredged materials but **also to upstream river sediments**, discharges of point sources included if possible. This seems reasonable for tracing back toxic potentials in dredged materials to its sources in the catchment basin as well as for the sake of comparability for the assessment of sediment quality. Some assays with marine species can be carried out with freshwater sediments but there might be the need to implement tests with fresh water species as well.
- Ø With regard to the interpretation of the results there is also the need for (1) guidelines of **sampling strategies** (number and distribution of representative samples)[2], (2**) standardisation and harmonisation of sampling**, e.g. for pore-waters and elutriates.

Question 2: *What are the current status and future approaches towards a decision-making framework for the disposal of dredged material?*

Current approaches for decision-making frameworks for the disposal of dredged material are schematically summarised in Figure 1. Focussing on the disposal in an aquatic system, e.g. coastal marine areas, it comprises the assessment of the dredged material (hazard assessment) as well as the risk assessment at the disposal site / receiving environment.

1. **Hazard assessment** includes:

- Defining hazard-levels for dredged material (potential risk)
- Deriving science-based quality criteria (SQC) on the basis of e.g. NOEC values

Currently different <u>approaches to derive SQC</u> for dredged material exist:

- equilibrium partitioning concept, i.e. wqg (water quality guideline value) x Kd, good criteria for water phase and translation to SPM and sediments
- spiked sediment toxicity tests
- co-occurrence analysis (matching the bulk sediment chemistry with effects observed in the field or in bioassays with in the field collected sediments)

2. **Risk assessment** includes:

- **Executing an overall impact study** (chemistry, toxicity, and benthic community) **at the disposal site / receiving environment**

Conclusions

- Ø The **decision making process** will be in the short-term as well as in the long-term **influenced by political decisions, resulting in <u>action levels</u>**. Generally decisions on the management of dredged material are driven by a combination of ecological and economic arguments (environmental yield / costs ratios).
- Ø Already defined **SQC** for individual chemicals **differ more than one order of magnitude**.

[2] Some ASTM guidelines already excist

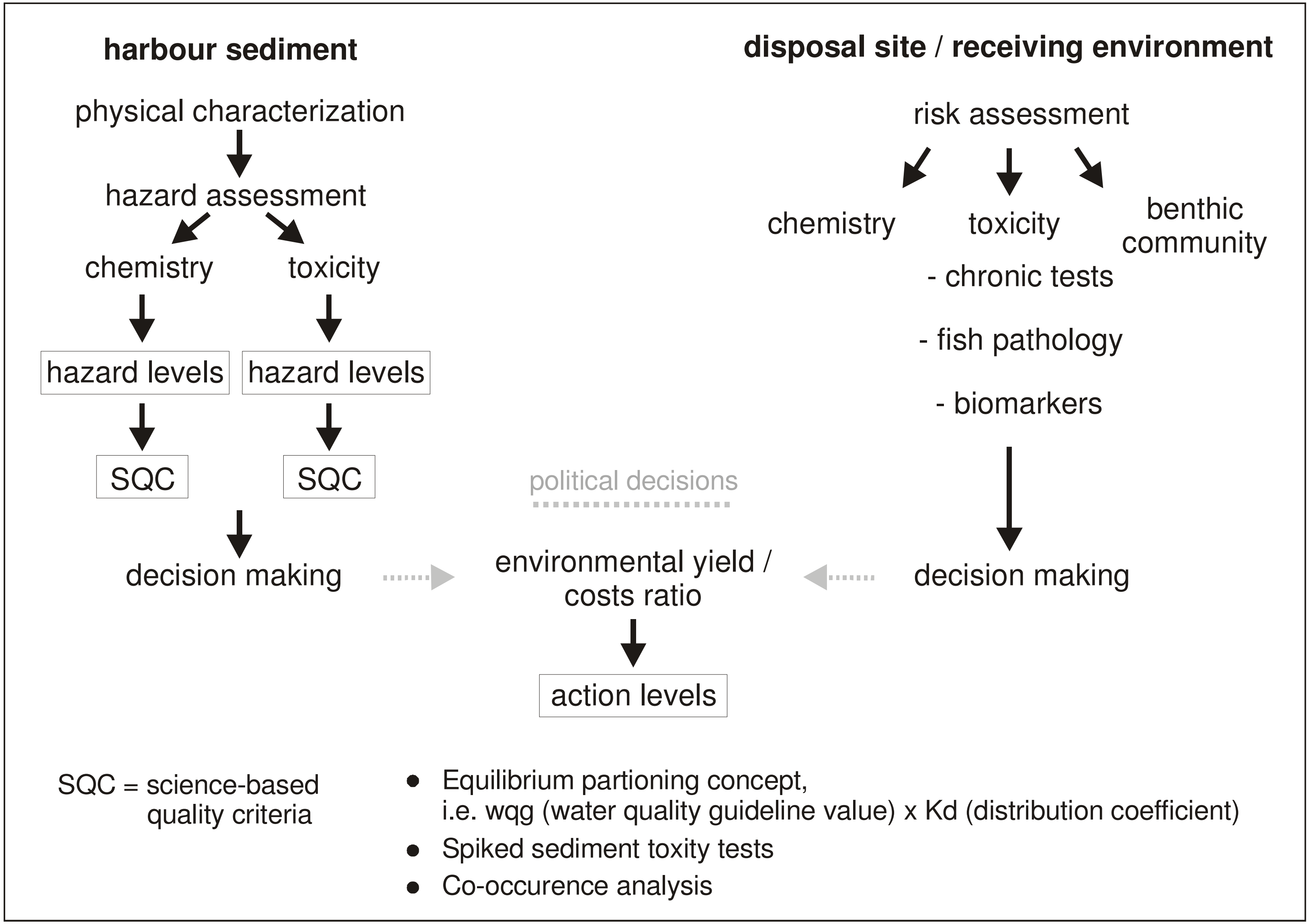

Figure 1: Decision-making framework for the disposal of dredged material

Recommendations

- Ø The **hazard assessment of dredged material** should be implemented in a **TIER-like approach**:
  - TIER I: limited chemical criteria, limited test battery with bioassays
  - TIER II: for toxic to highly toxic material for which the toxicity can not be explained by the investigated chemicals an **extended battery of bioassays** can be applied as well case studies undertaken to identify the culprit chemicals (e.g. **TIE approaches**).
- Ø **Standardisation and harmonisation** of deriving science-based quality criteria (**SQC**).
- Ø For a **risk assessment at the disposal site** in the marine environment **baseline studies** have to be undertaken including the disposal site itself as well as the effected area (dispersion of disposed/re-suspended material).
- Ø More effort has to be undertaken to make **the step from hazard assessment of dredged material to the risk assessment at the disposal site** / receiving environment (see also question 5: Translation of results from laboratory to field situations and vice versa).
- Ø **Monitoring the benthic community at the deposition site** can function as a **'safety net'** and might also have an impact on the modification of perhaps too stringent science-based quality criteria not taking into account other parameters like e.g. the coastal zone of the Southern Bight of the North Sea being a high dispersive environment. However benthic communities are often affected by physical impacts at the disposal site and it might be difficult to distinguish from impacts due to contamination.

Question 3: *Which methods could be used to assess sediment toxicity data?*

The decision-making process demands **reliable criteria which are easy to handle**. Currently there is no census for best methods to be applied. In the following list some methods are still under development or are visions for development (e.g. toxic units concept).

- Comparison with reference sediments
  In order to ensure statistical significance, dependent on the number of samples and allowed uncertainty, there should be at least 10 to 20% difference between dredged material and reference sediments, depending on the bioassay.
- Absolute classes
  - Power analysis (statistical derived min. limit value for classification in combination with eco(toxico)logically derived value, e.g. 25% mortality for amphipods acceptable with regard to the development of population)
  - Dilution factors for aqueous-phase tests (pore waters, elutriates, extracts)
  - Relative classes (e.g. TU, 'toxic units') which summarise results of different types of toxicity tests maybe even the chemical criteria into one yardstick ('toxic units concept'),
- Expert systems (research mode)
- Fuzzy logic based systems for classification

General recommendation

- Ø **Harmonisation** of methods should be approached in future (research mode, statistically sound and comparable databases as a prerequisite).

Conclusions for reference sediments

- Ø The use of reference sediments is already current practice, e.g. In the United States. Generally the statistical difference between dredged material and reference sediments is evaluated.
- Ø **Criteria for ideal reference sediments** are: (1) same grain size distribution and organic carbon content, (2) relatively uncontaminated in the sense of no response in applied toxicity tests, (3) as close as possible to the disposal site.
- Ø The **choice of sites for reference sediments is crucial** because it forms an integrated part of the decision-making framework (examples exist where decisions for the disposal of dredged material mainly depend on the choice of the reference sites).

Recommendations for reference sediments

- Ø Evaluation of the **original state at the disposal site** before disposal of sediments (often impossible as sites already have been used for a long time).
- Ø The **statistical evaluation between dredged material and reference sediments** is of high importance. In order to ensure statistical significance, dependent on the number of samples and allowed uncertainty, there should be at least 20% difference between dredged material and reference sediments).

Question 4: *What evidence can be obtained from results derived from experiments under laboratory conditions and in field situations? Can the results be translated?*

Figure 2 depicts tools for hazard/risk assessment which can be applied under laboratory conditions as well as under field conditions in a simplified manner.

Results from chemical analysis give amounts of single substances – the use of standardised and harmonised methods assumed – at a comparably high reproducibility in analysed samples. However their relevance to field situations in terms of negative effects to the aquatic ecosystem, not taking into account e.g. their bioavailibility, persistence, bioaccumulation etc., is low.

Field studies e.g. on benthic organisms have a high relevance with regard to possible impacts on the ecosystem but have a comparable low reproducibility. In between these two categories fall biomarkers, bioassays and mesocosm experiments.

The general problem is the translation of results between these categories of tools in either direction. This translation is essential in linking effects to toxic potentials to individual chemical substances and vice versa. In the case of biomarkers / receptor-based assays as the before-mentioned CALUX assay the results can be linked to the acting chemicals relatively straight forward. For most of the used bioassays this task is more complex. Promising tools for linking toxic potentials derived from bioassays to their causative chemicals – translating the results – are TIE-like approaches.

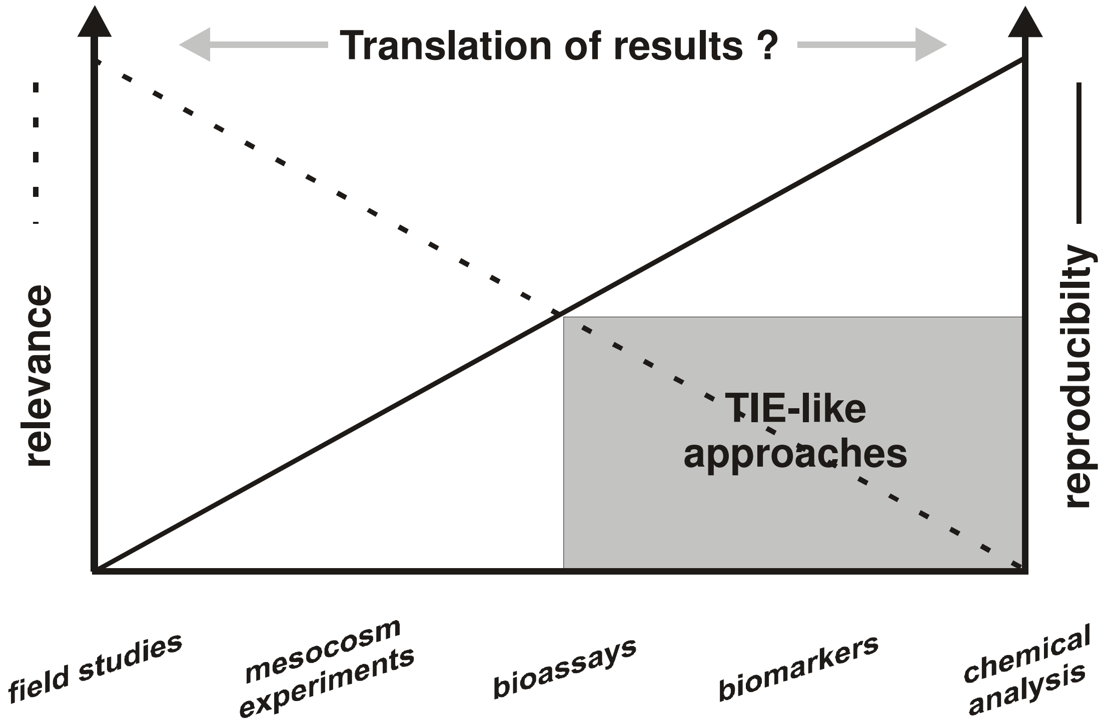

Figure 2: Translation from results derived under laboratory conditions and in field studies

Conclusion

Ø Without translation of results from chemical analysis, biomarkers, bioassays, (mesocosm experiments), and field studies the **link between effects and causative chemicals** as well as other influencing parameters can not be achieved.

Recommendations

Ø Efforts should be made to develop strategies for progressing **from hazard assessment of dredged material to the risk assessment at the disposal site**. This includes the necessity to improve translation of results as depicted in Figure 2.

Ø Advantages and disadvantages of **mesocosm studies** should be considered carefully before undertaking these studies at high costs.

Ø The application of **TIE-like approaches** is a reasonable tool under certain circumstances (see question 5).

Question 5: *Of what use are TIE-like approaches?*

The term **TIE** (Toxicity Identification Evaluation) refers to TIE protocols established by US-EPA. Similar approaches are described in literature where as well the **term bioassay-directed chemical analysis** was coined. They have in common that they combine chemical non-target analysis with toxicity tests with **the aim to identify the culprit chemicals**, i.e. identifying the chemicals which mainly contribute to toxic potentials.

Conclusions

Ø The identification of the **relevant chemicals** using TIE-like procedures is a prerequisite for **linking them to their emission sources** (first step for reduction measures).

Ø The **translation to field situations is not covered by TIE** approaches.

Ø The **obstacle** for TIE are **low or moderately contaminated** materials where the toxic potentials are dependant on a **broad spectrum of compounds** at low concentrations (mixed toxicity). Although the contaminants can be identified they might not be linked any more to their toxic potentials.

Recommendations

Ø TIE studies have relatively high costs and **should be applied in TIER II**, for dredged material/sediments (toxic to highly toxic) **where the toxic potential can not be explained by the identified chemicals** (chemical criteria in Tier I).

Ø TIE should be applied to **distinguish between toxic potentials from man-made and natural compounds** (e.g. phytoestrogens). This is comparable to the background approach for heavy metals.

Appendix I

Background Paper

distributed to the invited participants

Introduction

The management of dredged material in Europe is a major issue from its contamination, from the volumes which have to be dredged, as well as from the costs involved. Within the Rhine Research Project II of Rotterdam the long term issues of dredged material disposal are being considered.

Although in most river systems major results have been achieved in the reduction of point sources, there are still a number of substances for which the concentrations often exceed current quality criteria. Hence one of the aims of the project is to predict the future quality of the dredged material. In the case of the Rotterdam harbour this involves determining the major trends in the socio-economic developments in the Rhine catchment as well as to determine how future water quality regulations will affect point and diffuse sources.

In addition the regulations for dredged material disposal are subject to change, apart from pure chemical testing methods, biological assays are on the threshold of being implemented in certain countries.

Furthermore it has to be taken into account that these regulations, although implemented at the local level, increasingly become influenced by regulations/agreements at the European and international level.

Some of the issues of the workshop are:

- Within the policy field and also within the new “Water Framework Directive” of the EU there is more emphasis on ecosystem quality, flooding issues etc. and less on individual chemical substances. It is expected that new European regulations on water, air and soil quality will lead to further improvement of these environmental compartments. However, these regulations do not have dredged material quality as an objective. Translation of these regulations to expected sediment and as such dredged material quality is required. However, “dredged material” is not ranked high on the political agenda, despite the costs involved in their management at the European level.
- There is a strong shift in the Rhine catchment area from point sources to diffuse sources and very little is known for the Rhine catchment about the individual sources contributing to this diffuse load. A sound basis for predictions for the future or for establishing further reduction measures is still missing.
- There is a strong trend to complement chemical criteria with bio-assays for the assessment of dredged material quality. In a sense this is logical since there are more than 100.000 commercial substances (mainly organic) on the European market which simply cannot all be monitored in the environment. Hence the application of bio-assays seems logical but subject to pitfalls in their interpretation and a link with individual substances is generally lacking, i.e. that action towards the “polluter” is not possible. A complication with regard to the interpretation of the test results is that some dredged material may comply with criteria

based on chemical composition but not with the criteria derived from bio-assay techniques currently in use or vice versa.

- The present list of chemicals for assessing the quality of dredged material is limited and a number of new compounds are merging as likely candidates for inclusion in regulatory frameworks. Sources and pathways and their relevance for the quality of dredged material for these ‘new chemicals’ are yet largely unknown.

Most harbours in the North Sea region are situated at the end of a river catchment. Part of the fluvial sediments are deposited in the harbour, the other part is transported through the estuary to the coastal sea. In addition, through tidal action the harbours act as a sink for marine sediments also. Hence the deposited sediments are a mixture of original marine and fluvial sediments and are part of the (taking the holistic view) continuum catchment area-coastal sea. This view, which is not taken into account in current regulations, poses a number of additional questions:

- What makes the human pathway of transporting sediments to the coastal sea different from the natural pathway with regard to their impact?
- How do we translate the results of bio-assay methods of testing sediment quality at the deposition site (e.g. the catchment area) to a potential impact at the relocation site, which in the case of the North Sea can be a highly energetic environment? Here the sediments will partly remain at the site but subsequently will be dispersed and become part of the natural sediment movement along the coastline. Hence its properties both from a sedimentological viewpoint as well as of a toxicological viewpoint will be significantly altered from those in the harbour area.
- How do we link the results (bioassays and chemical criteria) to upstream discharges and diffuse sources which is needed as a basis for reduction measures.
- How are bioassay results of fresh water sediments are translated to the marine environment and vice versa?

During the workshop it won’t be possible to address all these questions. Hence one working group will focus on and evaluate bio-assays as a strategy for assessment of sediment quality and contaminant source control programs. The second working group will take a more holistic view with regard to the application of sediment quality criteria for the disposal of harbour sediments. The first day of the workshop is a symposium open to the general public. Lectures have been selected in such a way that the participants will obtain a broad overview on current and upcoming regulations with regard to dredged material with emphasis on national experiences on bio-assay techniques applied to contaminated sediments/dredged material.

Bio-assays as additional criteria for dredged material in the Netherlands

Over the past two decades the regulatory framework for disposal of dredged material was based on analysis of known classes of persistent chemicals, such as PCBs, heavy metals and mineral oil. The results of the analyses on these classes of chemicals are used as a guide for management of the dredged material, by comparing the results to existing normative levels at sea. In case the level of contaminants exceed these values, the dredged material is not licensed for offshore disposal, but in case of the port of Rotterdam, stored in a confined disposal facility (Slufter).

Recently, the above indicated practice of evaluating dredged material on the basis of chemical analysis results of only a limited set of contaminants is under debate. In fact, the regulatory authority is considering to add TBT to guide the management of dredged material. Moreover, it is realised that there may be thousands of other compounds and complex mixtures (e.g., xeno-estrogens) that are present at fairly low concentrations, but may still cause a hazard ,due to their high intrinsic potency. Therefore, a new concept for evaluating the level of contamination of river sediments, which is to be disposed at the North Sea, is under development. This concept consists of an integration of biological effect monitoring data and measurement of levels of chemical pollutants. In the 4th National Document on Water Management of the Netherlands it is stated that “measuring levels of just a limited number of selected compounds provides insufficient possibilities to estimate the impact of the complex mixtures of contaminants” In 2002 a system should be implemented that integrates bioassays with chemical analyses for assessment of the quality of sediments.

The bioassays that are being tested now for monitoring of river sediment toxicity, include laboratory tests where organisms are exposed to environmental samples. Three acute toxicity tests are at present under evaluation, namely a bacteria test, with a sediment suspension (Microtox (solid phase) and two whole sediment tests (the sea urchin *Echinocardium cordatum* and the amphipod *Corophium volutator*).. There is no direct indication by these acute toxicity bioassays of the culprit chemicals that may have caused the integrated effect. A fourth assay is the DR-CALUX that measures the overall exposure of compounds with a dioxin-like mode of action. These four bioassays together are valuable from a toxicology/ hazard identification point of view (since they give an integrated total impact assessment of whatever cocktail of chemicals is present in the sediments).

However, there are major limitations with regard to the management point of view of using acute toxicity tests alone as a management guide, since no indication can be derived from these bioassays on the chemical nature of the culprit chemicals causing the acute toxicity (both known and unknown chemicals have to be considered). In order to be able to effectively manage contaminated harbour sediment storage, handling and disposal in the near future there is a need to set up systems that would meet both the criteria of an integrative effect measurement, an effective way of identification of causative chemicals and identify the sources.

Items for discussion

Below a number of items are listed which can be relevant for and guide the discussion in the working groups.

- Are presently suitable bioassays or batteries of tests available which are already standardised (e.g. DIN/ISO, OECD) or are suitable for standardisation (other criteria are: relevance to the individual ecosystem, sensitivity, specifity, cost)?
- Is testing the *in-situ* quality of sediments at the deposition site necessary and can this presently be achieved on a sound scientific basis? Are beyond of chemical analysis of target compounds and the application of bio-assays ecological investigations to be considered as demanded by the OSPAR dredged material guideline?
- What is the relevance of using quality objectives for *in-situ* sediments when they are disposed off and dispersed in a high energetic environment?
- Can no-effect concentrations for the original dredged material be defined taking dispersion in the marine environment into account?
- How are the results of the bioassays quantitatively to be interpreted as guideline values for disposal (relocation)? E.g. some bioassays respond to certain non-anthropogenic (natural) substances or even to physical stress.
- Have new 'chemicals' (e.g. extended OSPAR-List, EC Water Directive) besides priority pollutants to be considered? Are these covered by the bioassays, e.g. endocrine disrupters?
- Is combining bioassays and chemical analysis (as US-EPA TIE and other bioassay-directed chemical analysis strategies) a suitable 'screening tool' to identify relevant 'new chemicals', i.e. substances that mainly contribute to toxicological potentials in dredged material?
- Can cause-effect relationships be established in order to launch substance-specific reduction programs for point and/or diffuse sources in river-catchment basins?

Appendix II

Workshop Agenda

Monday, April 3rd

10:00 - 10:30 Welcome and introduction to the workshop
G. von Sengbusch, Wim Salomons, GKSS, Germany

Session I Chair: Juergen Gandrass, GKSS

10:30 – 11:00 *Bioassays as screening tools for contaminated sediments*
Bram Brouwer*, IVM, The Netherlands*

11:00 - 11:30 The use and usefulness of bioassays to assess sediment quality: A case study of Hamburg Harbour
Susanne Heise, TU-HH, Germany

11:30 - 12:00 The status and use of bioassays for the assessment of contaminated sediments in the Netherlands
Joost Stronkhorst, RIKZ, Netherlands

12:00 –12:30 Use of bioassays in assessing the toxicity of dredged material: Experience in England UK
John Thain, CEFAS, United Kingdom

12:30 – 14:00 Lunch and poster break

Session II Chair: Ulrich Foerstner, TU Hamburg-Harburg

14:00 - 14:30 Effects based testing in the United States dredging program
Guilherme Lotufo, WES, USA

14:30 - 15:00 Setting toxicity criteria using multiple test endpoints: A comparison of multivariate and ranking methods
Lee Grapentine, CCIW, Canada

15:00 - 15:30 Selection and use of marine toxicity assays to assess the quality of dredged sediments
Marnix Vangheluwe, UG, Belgium

15:30-16:00 Federal regulations for the disposal of dredged material in German coastal areas – experiences with chemical and biological criteria
Birgit Schubert, BfG, Germany

16:00 – 16:15 Coffee break

16:15 - 17:00 Plenary discussion (Chair: Wim Salomons, GKSS)

Tuesday, April 4th

09:00 –9:15 The participants split up in two working groups

(1) Working Group I (science-oriented)
Chair: Bram Brouwer (IVM, The Netherlands)
Rapporteur: Susanne Heise (TU-HH, Germany)

(2) Working Group II (application-oriented)
Chair: Remi Laane (RIKZ, The Netherlands)
Rapporteur: Juergen Gandrass (GKSS, Germany)

09:15 – 12:30 Parallel sessions of the working groups

12:30 – 13:30 Lunch break

13:30 – 17:00 Parallel sessions of the working groups

Wednesday, April 5th

09:00 – 10:00 Presentation of preliminary reports from the two working groups (Susanne Heise, Juergen Gandrass)

10:00 – 11:00 Discussion in the plenum on the results of the working groups and the outcome of the workshop
Chair: Wim Salomons

11:00 - 12:00 Final discussion in the working groups

Appendix III

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