CEFAS
The Centre for Environment, Fisheries & Aquaculture Science

Marine Pollution Monitoring Management Group

The Group Co-ordinating Sea Disposal Monitoring

Final Report of the Sediment Bioassay Task Team

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Marine Pollution Monitoring Management Group

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Final Report of The Sediment Bioassay Task Team

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FOREWORD

The Group Co-ordinating Sea Disposal Monitoring (GCSDM), a sub-group of the Marine Pollution Monitoring Management Group (MPMMG), was set up in 1987 in order to co-ordinate the monitoring work carried out at sewage-sludge disposal sites. To help achieve its aims, the GCSDM established a number of specialist Task Teams to review the existing procedures used for the monitoring of sewage-sludge disposal sites and to define sediment quality criteria which could be employed to ensure that such waste disposal does not cause undesirable effects. In 1992, the Group's remit was extended to include a similar review of dredged material disposal sites.

Sediment Quality Standards for use at sewage-sludge disposal sites were developed, by the GCSDM Task Teams, for a number of metals and organic compounds, based on the Equilibrium Partitioning (EP) approach. Whilst this approach has proved effective for some chemicals, it has limitations, in that it fails to provide an adequate assessment of chemicals which have a low or non-existent aqueous phase toxicity. This is of particular relevance when assessing the potential toxicity of materials of unknown composition, such as dredged material.

At the time the GCSDM began its review of dredged material disposal sites, major advances were being made in the development of sediment bioassays. Bioassays can provide an integrated assessment of sediments such as dredged material, which is more difficult using a chemical-by-chemical approach. In view of this, in 1992, the GCSDM established a Sediment Bioassay Task Team to consider and recommend appropriate bioassays for use in evaluating dredged material prior to relocation.

In due course, the use of these biological techniques may supersede the numerical approaches developed by the Metals and Organics Task Teams (details of which will be published, in conjunction, in a further report in this series), but for the present it is recommended that both should run in parallel.

This document contains the final report of the Sediment Bioassay Task Team. It gives details of the Teams findings and the resulting recommendations as approved by the GCSDM.

SEDIMENT BIOASSAY TASK TEAM

FINAL REPORT
(Summary of work 1992-1994)

EXECUTIVE SUMMARY

The Sediment Bioassay Task Team was asked to consider and recommend appropriate bioassays for use in evaluating dredged material prior to relocation. The terms of reference of the Task Team were to review the availability and applicability of current sediment bioassay procedures and to recommend an approach and methods which could be applied to pre-dredging assessment of potentially contaminated sediments.

The following is a summary of the main findings and recommendations:

- Elutriate tests can provide useful data on the behaviour of sediment-associated contaminants during the disposal process, but are unlikely to provide information on the potential effects of contaminants retained on the solid phase and deposited on the seabed and are not therefore suitable as a primary tool for assessing suitability for disposal. The most suitable approach to testing dredged material is one which incorporates both elutriate and solid-phase testing. This will provide the most comprehensive information on the behaviour and availability of contaminants (both known and unknown) in the material.

- A number of existing aqueous-phase marine toxicity tests are considered to be suitable for testing elutriates. The most commonly used test species are: *Tisbe battaglia, Acartia tonsa*, *Crassostrea gigas* embryos and Marine algae (e.g. *Skeletonema, Phaeodactylum*). All of these tests are already routinely conducted in a substantial number of laboratories and are defined by well tested and widely-recognised guidelines or protocols. All are tests of short (24-48 h) duration, with broadly similar sensitivities to a range of toxicants.

- Two solid-phase bioassays are currently considered to meet the Task Team's requirements. These are procedures that use *Corophium* and *Arenicola.* Both techniques are capable of producing results from field sediment assays which are acceptably precise and consistent. Both methods have published guidelines (e.g. PARCOM, ICES) and have been ring-tested in a variety of contexts with acceptable results. Both methods are readily applicable to the direct bioassay of dredged material and the testing of sediment samples from the receiving environment and will thus enable the comparison of pre- and post-disposal effects in the same units of toxicity.

- For both methods the end point is mortality; a secondary endpoint in the *Arenicola* assay is a sublethal physiological response based on the rate of production of faecal casts on the sediment surface. A preliminary recommendation is to use the *Corophium* and *Arenicola* acute bioassay methods as initial tools for biological assessment of dredged material. In the medium to long-term, consideration should be given to the further development of the *Arenicola* sublethal assay and the development of comparable endpoints with crustaceans and molluscs.

- Reliance on a single test method is not recommended due to the risk of 'false negative' observations; the use of a 'battery' of tests is recommended.

- Assessment criteria (pass/fail) are by necessity largely arbitrary. Elutriate toxicity data should be interpreted in the light of estimates of mixing and dilution of the dredged material following disposal and in terms of the size of the defined mixing zone. For solid-phase dredged material tests, it is suggested that an initial criterion of 40%-50% mortality is adopted, but that this be modified in the light of the results of post-disposal solid-phase tests on the receiving environment. For solid-phase tests on the receiving environment, experience around offshore oil platforms suggests mortalities in excess of 20% can generally be regarded as an adverse effect of chemical contamination, though this experience needs to be extended to other environments.

1. INTRODUCTION

The Sediment Bioassay Task Team was convened to consider and recommend appropriate bioassays for use in evaluating dredged material prior to relocation. The team was established at a time when considerable activity was under way in the development and application of sediment toxicity tests and bioassays, and has to a considerable extent awaited the outcome of such developments.

Although bioassays and toxicity tests have been extensively used to determine toxicity thresholds for specific contaminants, a major perceived advantage of bioassays is that they can be applied to samples of unknown (or incompletely known) composition, and can provide an integrated assessment in a manner which can be difficult to achieve on a chemical-by-chemical basis.

The following document summarises the availability and applicability of current sediment bioassay procedures.

2. TERMS OF REFERENCE

The primary objective assumed by the task team was to recommend an approach and methods which could be applied to pre-dredging assessment of potentially contaminated sediments. Although there are potentially a large number of ways in which bioassays might be used to characterise dredged material, the parent body indicated that straightforward pass/fail criteria were desirable.

Confidence in pre-disposal assessment will be enhanced by evidence of its predictive reliability. Such evidence can be most readily obtained by means of post-disposal bioassays conducted on samples of sediment collected from the receiving environment. The application of the same bioassay method to pre- and post disposal samples permits a direct comparison which, while not providing a substitute for *in situ* biological assessment, enables an evaluation of the utility of the pre-disposal bioassay. The applicability of candidate methods is therefore considered in terms of both pre- and post-disposal monitoring.

3. ASSUMPTIONS

The approaches which can be taken to dredged material assessment depend on several assumptions:

- the primary aim of the team was to identify applicable generalised biological assessment tools, but that there may be an additional requirement to apply biological assessment to specific contaminants or classes of contaminant (i.e. bioassay techniques may be applied either as a stand-alone measure of environmental acceptability, or as a means of setting concentration standards for individual contaminants).

- evaluation will be carried out on sediment samples collected by grab or core from sites from which dredging occurs or is planned, and on samples of sediment collected from disposal areas.

- the statistics of sampling (location/collection/replication) will not be addressed specifically; sampling strategy should be designed to reflect the toxicological properties of the dredged material as it actually impacts marine sediments.

- sediment dredging may be carried out for engineering, navigational, or environmental reasons; dredged material evaluation may be conducted either:

  - to determine whether re-location is acceptable in relation to fixed criteria

  or

  - to enable a comparison of the costs and benefits of re-locating sediments or leaving them in place.

- environmental hazard may arise in two ways as a consequence of the release of dredged material at a disposal site

- contaminants may desorb to the water column, giving rise to transient effects on water quality

- contaminants which do not desorb may give rise to more persistent biological effects in material which settles on the seabed

- some contaminants may give rise to acute lethal effects, while the effects of other contaminants may only be expressed in sublethal terms over long time periods. This creates an immediate need for both a relatively rapid (i.e. acute) test which can readily distinguish between major classes of hazard (for preliminary screening purposes) and for a chronic (and sublethal) test which can reflect the effects of cumulative exposure during critical periods of an organism's life cycle.

- while dredged material may be derived from almost any aquatic environment (lake, river, estuary, sea), it is most likely to be re-located to a saline (estuarine or marine) environment and therefore the means of assessment should be based primarily on marine species

- recommendations should focus primarily on methods or practices which are currently achievable without significant technical development.

4. APPROACHES

4.1 Source of hazard

There is a clear distinction between hazard to the water column and hazard to sediment biota. The former is a transient phenomenon, limited in time and space to the desorption of contaminants as dredged material settles through the water column. The latter is likely to extend over longer periods, as contaminants retained in the dredged material will persist. In general, readily-desorbed materials will be less likely to accumulate at dredging sites, and are therefore less likely to present a hazard at sites of disposal. It is therefore considered that the primary objective of toxicological assessment of dredged material is to estimate the potential hazard in receiving sediments. Water column effects cannot, however, be dismissed as insignificant, and practicable methods for assessing such effects can usefully be recommended.

The disposal of dredged material will, in some circumstances, give rise to physical effects (smothering, alteration in sediment structure) which may have significant ecological effects independent of the effects of associated contaminants. This has been observed (and widely debated) in connection with the discharge of drill cuttings from offshore oil and gas operations, for instance; traditional field survey methods have been unable to distinguish between base oil toxicity, organic carbon enrichment, and sediment structure alteration as potential causes of benthic community alteration.

The incorporation of sediment bioassays into such field surveys has assisted in distinguishing the effects noted above, and indicates that, in the context of dredged material disposal, bioassays should be effective in helping to predict impacts over and above those of physical alteration of the habitat. The success of multi-disciplinary field surveys also provides some evidence that sediment bioassays can provide useful information from post-disposal monitoring.

4.2 Practical strategies

In assessing the hazard of dredged material to receiving sediments, a number of strategies are available:

(a) the toxicity of elutriates or chemical extracts may be assessed using standard acute aqueous phase bioassays;

(b) the toxicity of the whole dredged sediment may be assessed using an acute lethal or sublethal solid-phase bioassay;

(c) the toxicity of receiving sediments may be assessed using either lethal or sublethal solid-phase bioassays.

The use of elutriates may provide an effective means of ranking dredged material, but is more appropriate to the evaluation of water column effects during disposal - what is of primary interest in evaluating the *in situ* consequences of dredged material disposal are the effects of the contaminants which do *not* desorb from the material as it settles through the water column.

The objective of the task team has therefore been essentially to consider the availability and practicability of lethal and sublethal solid-phase assays, and the extent to which they can be applied to the assessment of dredged material prior to, and following, disposal. Lethal bioassays have been more extensively used and evaluated, but are likely to be inadequate for the assessment of contaminants which are of low acute/ lethal toxicity and are highly persistent; it is uncertain at this time, however, the extent to which sublethal assays are capable of addressing this problem (that is, to what extent they might reflect different modes of action or differ significantly in sensitivity from lethal assays).

During (and immediately prior to) the period of existence of the team, a considerable amount of activity has taken place in the area of sediment toxicity assessment. This has included multi-disciplinary field studies incorporating sediment bioassays, extensive evaluation of dredged material samples by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham laboratory, and a major international workshop on sediment toxicity assessment sponsored by Society of Environmental Toxicology and Chemistry (SETAC). Information from all these sources has been considered in formulating the recommendations provided below.

Existing approaches to dredged material assessment adopted by other regulatory agencies (e.g. United States Environmental Protection Agency (USEPA)), and outputs from other GCSDM task teams (e.g. the Organics Task Team) have also been considered and taken into account.

As a general proposition, it is considered that contaminant-specific biological assessment (e.g. 'safe' concentration thresholds for particular chemicals) are unlikely to be generically useful, because of the wide range of contaminant types, and the very large number of combinations of these contaminants which might be present in any given dredged material sample. Such thresholds will be useful primarily in situations where the presence and concentrations of contaminants of concern are known with certainty. Where doubt exists with respect to the contaminant load of a sediment, compliance with a particular set of concentration thresholds cannot guarantee an absence of unacceptable biological impact.

4.3 Regulatory examples

Notwithstanding the above, a number of regulatory authorities have adopted approaches which are based at least in part on the establishment of limiting criteria for chemical concentrations. It is perhaps worth explicitly recognising at this point that bioassays and toxicity tests are used in two distinct ways:

1. As a means of defining, for individual chemicals, a threshold or no-effect concentration which can subsequently be used to regulate discharge on the basis of chemical analysis.

2. As a means of establishing an integral biological criterion for effects and acceptability in whole sediments containing known or unknown mixtures of chemicals.

Existing approaches to the assessment of dredged material discharges, most notably in North America, tend to move from the first basis to the second basis at progressively 'higher' levels of evaluation. Two examples of detailed regulatory approaches are presented in the following sections.

4.3.1 US Environmental Protection Agency

The most comprehensive scheme developed to date was published in 1991 by the United States Environmental Protection Agency (USEPA) and the United States Army Corps of Engineers (USACE), and is commonly referred to as the 'Green Book' (USEPA/USACE, 1991). This manual contains technical guidance for determining the suitability of dredged material for ocean disposal through chemical, physical and biological evaluations. Integral to the approach is a tiered testing procedure for evaluating compliance with the limiting permissible concentrations (LPC) as defined by the United States Ocean Dumping Regulations. The procedure comprises four levels of increasing investigative intensity. Tiers I and II utilise existing or easily-acquired information and apply relatively inexpensive and rapid tests to predict environmental effects. Tiers III and IV contain biological evaluations which are more intensive and require field sampling, laboratory testing and rigorous data analysis.

All laboratory-generated data are compared with a reference sediment which is similar in characteristics to the natural sediment of the dredged material disposal site.

Tier I
The purpose of Tier I is to identify the contaminants of concern by collecting and analysing existing data. At this stage, the following concerns are addressed:

- known current and historical sources of pollution;
- time since historical sources remediated;
- frequency of dredging (past and future);
- mixing and dilution;
- quantity and toxicological importance of contaminants.

Chemicals of concern are identified on the basis of presence, toxicology, persistence, bioaccumulation, hydrophobicity, aqueous solubility, stability and stereochemistry. At this stage, there may be a requirement for confirmatory analysis for chemicals of concern, and if no existing information is available then bulk sediment chemical analysis is conducted. The 'Green Book' contains comprehensive lists of detailed information which is required at this stage, together with a list of chemicals identified as of concern to the USEPA.

Tier II
This tier consists of:

- A model to determine compliance of chemical concentrations with marine water quality standards. The model assumes as a 'worst case' that all of the contaminant of concern will partition into the water phase. If the predicted concentration exceeds the relevant water quality standard, then elutriate tests are carried out and the concentrations of eluted chemical measured. If there are no standards available, or if synergistic effects are suspected, then the sediment must undergo toxicity testing in Tier III.

- A calculation of theoretical bioaccumulation potential (TBP) based on the partitioning of contaminant between the organic carbon phase and the lipid in the organism. If the TBP exceeds that of a reference sediment then a laboratory bioaccumulation study is required.

Tier III
This tier consists of:

- Water column testing - this must be carried out if no water quality standards exist for the chemicals of concern or if synergistic effects are expected. This stage considers the effects, after allowance for initial dilution, of dissolved contaminants plus those associated with suspended particulates on water-column organisms. Tests must include a specified range of ecological and taxonomic groups, examples of which are provided in the guidance document.

- Benthic bioassays - benthic impact is evaluated by comparing dredged material toxicity against reference sediment toxicity. Tests must again cover a range of ecological and taxonomic groups. Short-term acute lethal tests (10 days) are carried out exposing test organisms to whole dredged sediment. The LPC is not met if the dredged material toxicity is statistically greater than that of the reference sediment and exceeds reference sediment toxicity by at least 10-20%.

- Bioaccumulation testing - bioaccumulation is evaluated by comparing bioavailability of contaminants in the dredged material with action levels in the Food and Drug Administration (FDA) regulations.

The document provides detailed guidance for this tier in respect of

- collection and preservation of samples;
- sampling design - selection of sites and number of samples;
- water sample collection;
- organism collection;
- sample handling, preservation and storage;
- physical and chemical analysis.

Tier IV
Testing in this tier is case-specific and is intended for infrequent application under exceptional circumstances. Rigorous toxicity testing and steady-state bioaccumulation tests are recommended.

4.3.2 Ontario Ministry of the Environment and Energy

The Ontario Ministry of the Environment and Energy (MOE) has developed a protocol for establishing sediment quality guidelines which include criteria for dredged material disposal (MOE, 1993).

The MOE approach is based more exclusively on chemical concentration thresholds than the USEPA approach; No-effect, Lowest effect, and Severe effect levels are derived in the following way:

No Effect
This is designed primarily to protect against biomagnification through the food chain, and is derived using partitioning models normalised for organic carbon content.

Lowest Effect Level
This is the level at which ecotoxicological effects are expected to become apparent. MOE examined a variety of approaches based on the empirical comparison of field concentrations of chemicals with observed biological effects, and selected the Screening Level Concentration (SLC) as the most useful current approach. The SLC uses field data on the co-occurrence in sediments of benthic infaunal species and different concentrations of contaminants. The SLC is an estimate of the highest concentration of a contaminant which can be tolerated by a specific proportion of benthic species (for instance, 95%).

Severe Effect Level
This is the level at which the SLC approach would predict that 95% of benthic species would be eliminated.

Interestingly, although MOE considered the use of chemically-spiked sediment toxicity tests as a tool in determining the potential hazard of individual chemicals, the use of whole sediment bioassays as a preliminary tool to evaluate the overall toxicity of sediments was not considered. The published scheme requires a sediment bioassay only *after* a sediment has been shown to exceed specified concentration thresholds for identified contaminants.

Decisions on the disposal of dredged material are based on the comparison of the Effect Level of contaminants in the source and receiving sediments, and essentially permit the relocation of sediment to an area of equal or higher Effect Level, while prohibiting relocation which would downgrade the classification of the receiving sediment.

5. METHODS

5.1 Elutriate tests

Elutriation is defined as the process of equilibrating a volume of sediment with a volume of diluent (i.e. in the present context, seawater) for a specified period of time, after which the diluent is separated (by settling, filtration or centrifugation) and subjected to an aqueous phase toxicity test.

As indicated above, elutriate tests are, by definition, unlikely to provide information on the potential effects of contaminants retained on the solid phase and deposited on the seabed. They are a useful tool, however, in determining the extent to which contaminants may be firmly associated with dredged material, and therefore the extent to which concern should be focused on water-column or seabed effects. Where significant toxicity is associated with elutriates, it might be advisable to conduct solid-phase tests on the material after elutriation, to determine whether settled material poses any residual hazard.

Procedures for elutriation vary widely in respect of the relative volumes of solid and liquid, the contact period, the mixing energy supplied, and the means of separating the liquid and solid phases. Ideally, elutriates should be prepared at a range of sediment:water ratios, to permit an assessment of the minimum rate of addition required to elicit a maximum response. This direct addition approach is preferable to the preparation of serial dilutions from a stock preparation at a single sediment:water ratio. Settlement or centrifugation of the mixture is preferable to filtration in preparing a final exposure medium. In this respect, it is probable that the test methods listed below will differ significantly in the extent to which they can tolerate residual suspended (i.e. colloidal) material. The degree of tolerance should be quantified as far as practicable before extensive use is made of any test method.

It is considered that a number of existing aqueous phase marine toxicity tests are adequate for testing elutriates. The most commonly used test species are:

Tisbe battaglia
Acartia tonsa
Crassostrea gigas embryos
Marine algae (e.g. *Skeletonema, Phaeodactylum*)

All are tests which are routinely conducted at present in a substantial number of laboratories, and which are defined by well-tested and widely-recognised guidelines or protocols. All are tests of short (24-48 h) duration, with broadly similar sensitivities to a range of toxicants.

It should be emphasised that, while elutriate tests may provide useful ancillary information about the behaviour of sediment-associated contaminants during the disposal process, they should not be used as a primary tool in assessing suitability for disposal. In some respects, the sediments which might be of greatest concern would be precisely those which did *not* give rise to measurable elutriate toxicity - these could be either innocuous or contaminated with highly persistent chemicals.

The most useful approach to testing dredged material would be one which incorporates both elutriate and solid-phase testing, since this would provide the most comprehensive information on the behaviour and availability of contaminants (both known and unknown) in the material.

5.2 Partitioning models

The availability of extensive toxicity databases for chemicals in the aqueous phase has provoked an interest in many countries in finding ways of using these data to predict sediment toxicity. The principle is attractive, since it would enable sediment quality criteria to be established for a large number of chemicals without the need to conduct additional solid-phase tests. The approach has been pursued with both organic chemicals, trace metals, and organometallic complexes, and considerable progress appears to have been made in identifying the major physico-chemical attributes of sediments responsible for controlling partitioning and (it is assumed) toxicity.

There would, in principle, be substantial advantages to being able to predict sediment toxicity potential from existing routine chemical assays, if sufficient aqueous phase toxicity data were available for the contaminants of concern in any given situation.

The most widely-considered approach is based on the assumption of equilibrium partitioning of chemicals or metal species between sediment particles and interstitial water. Given this assumption, porewater concentrations may be estimated from bulk sediment concentrations, and subsequently compared with aqueous phase toxicity data to predict sediment toxicity. While the equilibrium partitioning (EP) approach has been 'validated' for some non-ionic organic chemicals, and has been tentatively applied to some organometals, it has not yet been generalised to a wide range of chemical types. This limits the utility of the approach in assessing sediment prior to disposal.

A more fundamental limitation is that, while the chemical theory underlying the partitioning models is not in question, there is evidence that the models may be applied to an inappropriate 'physical' model. Evidence is becoming available indicating 'exceptions' to the EP approach - that is, organic chemicals which at saturation aqueous concentrations have low or negligible aqueous phase toxicity prove to be highly toxic in sediments. Since the porewater concentration cannot reasonably be expected to exceed saturation concentrations, it is apparent that, while the porewater may be the *route* of exposure, the porewater *concentration* cannot be directly predictive of toxicity.

The EP approach does not, at present, take account of the role of dissolved organic carbon and ingestion in mediating exposure to, and uptake of, contaminants.

The risk inherent in the EP approach at present is that, while it may be applicable to a number of highly persistent chemicals with well-studied properties, it may fail to be adequately protective of the environment for hydrophobic chemicals which are of low or non-existent aqueous phase toxicity. The corollary of this is that the approach is directional: it may tell us whether or not chemicals which have measurable (i.e. quantifiable) aqueous phase toxicity are likely to be toxic in sediments, but it cannot tell us anything useful about the sediment hazard of the complementary set of chemicals.

In general there is a lack of literature data for the EP approach in the actual situation in the marine environment, where all the variables affect the equilibria, and are subject to simultaneous changes. Also, in real processes occurring in the environment equilibrium does not always exist due to the heterogeneity and hydrodynamics of the system.

As such the EP approach has theoretical merit but in reality, assessing whole sediment toxicity and bioavailability of contaminant-bound sediments, can only be achieved by direct exposure of organisms to the sediment.

5.3 Solid-phase tests

Considerable activity has taken place in the past three years in the development and evaluation of solid-phase sediment bioassays and toxicity tests, culminating in the recommendations of a workshop held in Renesse in November 1993 (SETAC, 1993) under the auspices of SETAC and with the objective of publishing a guidance document on test method design. The workshop established that methods had been developed to the point where assays could be considered practicable and reliable. In respect of marine bioassays and toxicity tests, the workshop endorsed the prior recommendations of the Paris Commission (PARCOM) that assays with the amphipod *Corophium* and the polychaete *Arenicola* could be recommended for routine use. Well-defined protocols for testing with both species were submitted to PARCOM, and have been successfully implemented by a substantial number of laboratories.

The test methods referred to above have been increasingly widely used as sediment bioassays (i.e. to assess the toxicity of contaminated field sediments) as well as toxicity tests for individual chemicals. Both the *Corophium* and *Arenicola* tests are based on a 'generic' test design derived from American Society for Testing and Materials (ASTM) guidelines (ASTM, 1993); static exposure (i.e. no replacement of water or sediment) in simple containers (*c.* 1 litre) for a period of ten days. An essentially similar design has been used extensively in North America with the amphipod *Repoxynius abronius*, and in Europe with the urchin *Echinocardium.*

While it is, in principle, feasible to adapt the generic design to permit the conduct of chronic (long-term) or sublethal toxicity assays, the bulk of studies carried out to date have used mortality as the primary endpoint. Both the *Arenicola* and *Echinocardium* tests include sublethal endpoints (feeding or burrowing behaviour), but field data for these endpoints are less extensive than for lethal tests (in both Europe and North America).

Bearing in mind the constraints noted above in the use of elutriate tests and partitioning models, there are sound *a priori* reasons to examine the merits of basing dredged material assessment on solid-phase tests. Although, as with any measurement system, such tests will be unlikely to 'accurately' reflect fundamental biological properties of sediments, they appear to introduce less bias, and make fewer assumptions, than the other approaches described in earlier sections. What is not known with any confidence at present, is the extent to which bias is introduced by the process of sampling and processing a sediment sample for testing.

6. METHODS RECOMMENDATIONS

The foregoing discussion provides a basis for recommending solid-phase bioassays as the primary tools for biological evaluation of dredged material. It should be recognised, however, that elutriate tests are a valuable complement to solid-phase tests, and can provide important additional information on the availability and behaviour of contaminants.

To be of practical value, candidate methods must satisfy general requirements not addressed specifically above *viz*:

- be in routine use in UK laboratories;
- be readily transferable between laboratories, and of reasonable cost and simplicity to implement;
- have been applied to the evaluation of contaminated sediment in UK waters, with a satisfactory response range;
- involve test organisms that are readily available and tolerant of varied sediment types.

6.1 Elutriate methods

The water-phase methods listed in Section 5 are all considered to meet the above criteria, and have all been applied successfully to elutriates.

6.2 Solid-phase methods

6.2.1 Acute lethal tests

At present, two solid-phase bioassay methods meet the requirements listed above. These are the procedures which use *Corophium* and *Arenicola*. There is no absolute basis for distinguishing between these methods in terms of their suitability; both techniques are capable of producing results from field sediment assays which are acceptably precise and consistent. Both methods have published guidelines (e.g. PARCOM, 1994, Thain *et al*., 1994) and have been ring-tested in a variety of contexts with acceptable results.

For both methods the primary endpoint is mortality; a secondary endpoint in the *Arenicola* assay is a sublethal physiological response based on the rate of production of faecal casts on the sediment surface. Studies on dredged material samples conducted by the CEFAS Burnham laboratory, which compared *Corophium,* *Arenicola* and elutriate tests registered a higher number of 'positive toxic' responses with the *Arenicola* test than with other methods.

Field sediment studies have been conducted routinely in the UK by a number of laboratories, including CEFAS, Environment and Resource Technology (ERT) and Shell Research, and in several instances collaborative programmes have permitted intercalibration of *Corophium* tests on 'identical' sub-samples. The results of intercalibration exercises (coastal and oil-platform monitoring) have indicated good reproducibility for this test method, and have also provided a basis for suggesting empirical 'detection limits' - i.e. levels of response below which toxicity cannot be confidently inferred. On the basis of several paired comparisons, the above laboratories suggest that a provisional detection limit of 20% mortality be adopted; although statistical analysis may on occasion indicate that far smaller effects are significant (i.e. significantly different from controls), variation in control response from time to time requires a more conservative threshold to establish a consistent baseline for evaluation. The *Corophium* procedure is routinely in use as a toxicity test and bioassay in at least eight laboratories in the UK.

Corophium bioassays have been conducted on North Sea sediments in the vicinity of oil platforms in conjunction with nine comprehensive biological/ chemical surveys in the past five years. The data generated, although not yet all in the public domain, have demonstrated a consistent ability to reflect contamination gradients, and show a high degree of correlation with indices of community response. This indicates that, in practice, lethal effects in bioassays can be elicited at environmentally-realistic concentrations of some types of contaminant (heavy metals and hydrocarbons). Insufficient data are available to enable this observation to be generalised to all, or even the majority, of anthropogenic contaminants.

The experience of CEFAS, and other organisations, is that reliance on single test methods should be avoided if possible, due to the risk of 'false negative' observations. It is therefore recommended that the principle of using a 'battery' of tests be adopted.

A preliminary recommendation is to use the *Corophium* and *Arenicola* acute bioassay methods as initial tools for biological assessment of dredged material. These methods at present best satisfy reasonable criteria. In the medium to long term, consideration should be given to the further development of the *Arenicola* sublethal assay, and to the possibility of applying a basic, generic solid-phase test method with other taxa, to provide an extended and more flexible means of controlling for 'false negative' results.

Both methods are readily applicable to:

- the direct bioassay of dredged material;
- the testing of 'dilutions' of dredged material in clean sediment
    - this may be an appropriate strategy if dredged material samples are highly toxic but are to be disposed of in small quantities;
- the testing of sediment samples from the receiving environment
    - it should be emphasised in this respect that the primary objective is to compare like with like (that is, to compare pre- and post-disposal effects in the same units of toxicity), and not to substitute receiving sediment bioassays for ecological assessment of receiving habitat quality.

6.2.2 Sublethal and bioaccumulation tests

The potential environmental consequences of contaminants which have chronic effects (such as polychlorinated biphenyls (PCBs)) will not readily be detected by the use of acute lethal assays, and the development of the latter type of test is considered an essential future step in the application of bioassays to dredged material assessment. As noted above, the *Arenicola* test already incorporates a measure of sublethal effect, and it is recommended that consideration be given to the development of growth, development or feeding rate test methods for other infaunal organisms (for instance; growth and development in *Corophium* juveniles, feeding rate in tellinid bivalves such as *Macoma, Abra* or *Scrobicularia*).

A concomitant feature of chronic contaminant impact is that sublethal toxicity will often be a result of long-term bioaccumulation, and the development of a bioaccumulation test (using a sediment-dwelling deposit-feeder such as *Macoma*) would provide an effective means of identifying potential sources of chronic effects.

7. PROCEDURES AND CONSTRAINTS

7.1 Sampling strategy

It has been noted above that field sampling design should initially comply with existing practice, to ensure that biological and chemical assessments are comparable. It should also be noted, however, that a definitive strategy for dredged material sampling has not yet been finalised.

It is recommended that, wherever practicable, samples for bioassay purposes be taken synoptically with those collected for chemical analysis; ideally, samples should be taken from the same grab. Existing practice may not, however, adequately reflect the spatial heterogeneity of dredged sediments, and it will be important to ensure that bioassay procedures keep pace with developments in sampling strategies for chemical analysis.

It is recommended that bioassay samples be taken from a thoroughly homogenised grab sample, ensuring that at least the top 20 cm is well-mixed (when taking samples for routine bioassays, it is normal to sample the top 2-4 cm only). Samples should be placed in polythene bags, as much air as possible excluded, and the samples chilled immediately.

The bulking and homogenisation of a number of sub-samples from each site is a procedure which would more closely reflect the actual dredging process.

Opinion varies with respect to sample storage. There is a limited amount of evidence to support and challenge the idea that freezing sediments alters their toxicological properties. Whilst it is very likely that changes will occur, it is equally likely that sediments stored at 4°C will also undergo change with time. If samples are to be stored for extended periods, then it may be advisable to 'standardise' the effect by freezing, since subsequent changes will be minimised.

It would be preferable to conduct comparative trials for a range of dredged material samples to resolve storage uncertainty by measurement.

Whether samples are chilled or frozen, it is essential that large indigenous organisms be physically removed before commencing a bioassay. Sieving should be avoided if possible, and it is preferable to remove organisms by hand. The presence of indigenous biomass is likely to constitute an interference, for instance by predation on test organisms (if the indigenous animals survive storage) or by generating excess biological oxygen demand (if they die and decompose).

7.2 Sediment avoidance

Occasionally, it may be difficult to assess the toxicity of sediment samples because the test organisms avoid the substrate completely. For extremes of sediment type, this may be related to the ability of the animal to actually penetrate the substrate; in other cases, it is likely to be due to some chemical property of the sediment, either associated with toxicity or with 'palatability'. Avoidance of sediments by *Corophium* and *Arenicola* has not been widely observed, but where it occurs it is recommended that the sediment sample be tested by serial dilution with a clean reference (control) sediment. This will, at least, permit a determination of the degree of dilution necessary to encourage entry into the sediment; if biologically active contaminants are present, it may also ensure sufficient exposure to observe and measure toxicity.

7.3 Severe toxicity

Circumstances may arise in which dredged material samples exhibit severe toxicity, but may be associated with very limited dredging and/or with disposal at a highly dispersive site. In such cases, it may again be useful to conduct tests on serial dilutions of the dredged material in clean sediment, to establish whether there might be an acceptable rate of application or deposition of the dredged material at the disposal site.

8. PROPOSED ASSESSMENT CRITERIA

Assessment criteria (pass/fail marks) are of necessity largely arbitrary. It was noted above that, for the *Corophium* test, 20% mortality was deemed to be a reasonable definition of the detection limit - mortality rates below this level cannot consistently and with confidence be attributed to toxic effects.

It has also been noted that sediment bioassays can produce results which are broadly in proportion to observed *in situ* community effects. It was felt, in discussion, that a threshold of 40-50% mortality would be a reasonable starting point for a pass/fail criterion; this would allow for a 2-fold 'dilution' of the material when incorporated into receiving sediments without risk of interference at the 'detection limit'. A sediment sample, undiluted, which elicited a 40-50% response might be expected to cause impaired biological quality of the receiving environment, i.e. the sea and just outside the disposal area.

Assessment criteria for post-disposal monitoring can be provisionally based on the findings, referred to above, from field studies conducted in the North Sea. In acute lethal bioassays of sediments, mortality in excess of 20% appears to be consistently related to observable impairment of biological communities in the vicinity of oil platforms. The extent to which this finding can be definitively applied to dredged material disposal sites has not yet been demonstrated.

Assessment criteria for elutriate tests would be most appropriately set in the context of mixing and dilution estimates; with adequate modelling, it should be practicable to establish criteria for 'no effect' at the edge of a defined mixing zone.

9. VALIDATION

The foregoing recommendations represent a starting point which is practicable under present circumstances. Recommendations do not, however, provide a guarantee of satisfactory performance. It is considered desirable to validate the proposed approach, and a practicable means of accomplishing this would be to invite participation in a 'ring test', in which laboratories would conduct standard tests on samples of dredged material distributed for the purpose. This exercise would provide a sound basis on which to establish confidence limits for pass/fail criteria, without which decisions on values close to the boundary will inevitably be somewhat arbitrary.

10. RECOMMENDATIONS

1. Principles

Bioassays can provide both a means of establishing contaminant-specific effects threshold concentrations and a means of integrally assessing the relative hazard of complex mixtures of contaminants.

It is considered desirable to employ a battery of test methods wherever practicable. Reliance on a single test method is likely to result in failure to detect some potentially harmful materials.

2. Types of test

Elutriate tests are considered to be a valuable component of dredged material assessment.

Solid-phase, lethal tests with *Corophium* and *Arenicola* are recommended as immediately practicable components of a battery of test methods. Published protocols are available for both methods, and both have been extensively employed by UK laboratories as bioassays and toxicity tests.

3. Application of tests

Water-column
Elutriate tests should be employed to evaluate the behaviour of weakly-sorbed contaminants, and to assess the extent to which sediments might release contaminants shown to be toxic in the solid phase.

Dredged material
Solid-phase tests, as identified above, can practicably be employed on homogenised whole, or 'diluted' dredged material samples as a means of ranking and comparing samples between times and sites.

Receiving sediment
Solid-phase tests, as identified above, can be used as direct bioassays of receiving sediments, to provide a quantitative basis for refining and improving toxicity pass/fail criteria.

4. Methods development

Existing lethal methods are unlikely to respond to chronic, accumulative contaminants such as PCBs. There is a need to develop sublethal assays for this purpose. Sublethal endpoints with *Arenicola* should be complemented by the development of comparable endpoints with crustacea and molluscs.

5. Pass/fail criteria

Elutriate tests
Elutriate toxicity data should be interpreted in the light of estimates of mixing and dilution of the dredged material following disposal, and in terms of the size of acceptable mixing zone thus defined.

Solid-phase dredged material tests
It is suggested that an initial criterion of 40-50% mortality is adopted, but that this be modified in the light of the results of post-disposal solid-phase tests on the receiving sediment.

Solid-phase tests on receiving sediment
Experience of testing sediments collected from around offshore oil platforms suggests that mortalities in excess of 20% can generally be regarded as an adverse effect of chemical contamination though this experience needs to be extended to other environments.

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