EEDP-01-40
August 1996

Environmental Effects of Dredging Technical Notes

Assessment of the Genotoxic Potential of Dredged Material

Purpose

This technical note describes an approach for assessing the genotoxic potential of dredged material. The use of integrated batteries of rapid and mechanistically interpretable *in vitro* and *in vivo* assays in a tiered approach is fundamental to applied toxicology. The research described here brings this approach to the testing of sediments. Work completed to date and future work will mesh to form an advanced and cost-effective methodology. The purpose of this methodology is to increase the accuracy of environmental risk assessments and facilitate making decisions concerning open-water disposal of dredged material.

Background

A great number of the contaminants typically found in dredged material are toxic to exposed organisms through effects on DNA. Such effects are usually the result of low-level chronic exposures. These effects can result in reproductive failure of organisms, impaired growth and development of offspring, and tumors (often cancerous) in vertebrates. Collectively, such effects are called "genotoxicity" and result from damage to the genome of a cell. The damage is heritable, that is, passed on to future cell generations upon duplication of the affected cells.

Although tests of sediment genotoxicity are not routinely applied in regulatory contexts, the potential for their requirement in special circumstances is implied by the language of U.S. public law. For example, Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532), which regulates disposal of dredged material in coastal regions, specifically

prohibits open-water disposal in other than trace amounts of "known carcinogens, mutagens, or teratogens or materials suspected to be carcinogens, mutagens, or teratogens by responsible scientific opinion." In addition, the emphasis in environmental toxicology over the last decade has increasingly shifted away from the catastrophic end point (death of individual organisms in acute exposures) to chronic and sublethal effects that have long-range potential to seriously affect the viability of populations of organisms. To be accurate, risk assessments involving environmental contamination must take genotoxic potential into account.

Additional Information

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Approach

A tiered approach is being developed in which a battery of mechanistically related, rapid, low-cost assays are applied initially. Based on the results of these assays, decisions can be made as to whether more definitive tests are necessary at higher tiers in the evaluation. The assays are based on the approach of the U.S. Environmental Protection Agency's Health Effects Research Laboratory for assessing the genotoxic potential of chemicals to rodents and humans (Kitchin, Brown, and Kulkarni 1994).

The battery contains two types of tests: assays to assess damage to DNA and assays to assess nongenotoxic adjuncts of DNA damage. The rationale for selecting these two types of assays lies in the knowledge that cancer and other results of DNA damage are multistage events requiring alterations in protein synthesis and cell development and function. For example, the development of cancer involves processes known as initiation and promotion. Initiation can be simply defined as damage to DNA, also known as mutation. A mutation occurs when a DNA nucleotide is chemically modified, deleted, or substituted. Certain environmental contaminants act as mutagens in that they covalently bind to DNA nucleotides, chemically modifying the DNA. The cell contains DNA repair enzymes that can repair mutations under normal circumstances.

When the organism is exposed to an excessively high level of a mutagen, the DNA repair enzymes may not be able to repair all of the mutations or may misrepair some mutations by deleting the nucleotide rather than replacing it, or by substituting a wrong nucleotide for the mutated one. Depending on the location of the mutation, the number of mutations, and whether the mutation is repaired by the cellular DNA repair enzymes, a mutation may progress to tumor formation or cancer in the organism. The stage of cancer development following initiation is promotion, in which the initiated cell is

altered to allow reproduction of the cell, passing the "defect" on to daughter cells.

To adequately assess dredged material genotoxic potential, the ability of sediment contamination to cause DNA damage and its subsequent effects on exposed organisms must be ascertained. Even if analytical chemistry were capable of identifying and quantitating all the genotoxic agents present in a sediment, an assessment of genotoxic potential could not be made with analytical data alone because contaminants interact in unpredictable ways. The toxicological approach involves the use of a battery of biomarker-based *in vitro* assays on sediment extracts in the first level, or tier, of testing. These assays assess the potential for DNA damage and the subsequent biochemical and molecular changes that lead to tumor formation and other adverse somatic effects. The second level of testing is *in vivo* testing, which involves exposing fish to the dredged material and assessing genotoxic effects, thereby incorporating bioavailability of sediment-associated contaminants.

In Vitro Testing

In vitro testing uses two basic types of assays for mutagenicity. Bacterial assays (Ames test and Mutatox) are designed to detect the presence of mutagenic compounds in a sample. A second type of assay (alkaline unwinding) is used to determine whether an exposed living cell has experienced mutations.

The *in vitro* testing battery also uses tests of nongenotoxic effects on adjunct systems. These assays include cytochrome P450 induction, glutathione fluctuations, ornithine decarboxylase activity, oxidative stress, and cytotoxicity. Cytochrome P450 is a family of enzymes found in most living organisms and is primarily responsible for metabolism of environmental contaminants. Exposure to certain classes of genotoxic compounds (for example, polycyclic aromatic hydrocarbons (PAHs), dioxins, and polychlorinated biphenyls) induces the formation of cytochrome P450, which has a promotional effect on initiated cells. Glutathione is a small peptide that functions as the major defense against electrophilic compounds in most vertebrate organisms. Electrophiles bind to DNA and thereby cause mutations. An organism (or cell) may be depleted of glutathione upon exposure to such compounds, leaving it vulnerable to an increased rate of mutation.

Ornithine decarboxylase is an enzyme that, when present, indicates cellular proliferation and signals possible exposure to a cancer promoter. While oxygen (O_2) is essential for life functions of all multicellular organisms, some forms of "reactive oxygen" produced during metabolism (for example, superoxide anion radicals ($O2\cdot^-$), hydroxyl radicals ($OH\cdot$), and hydrogen peroxide (H_2O_2)) are highly reactive and can damage DNA. Subcellular biochemical changes such as these can also lead to cytotoxicity, or cell death. All of these biomarkers can be measured *in vitro* and, when used together, provide a short-term means of predicting carcinogenicity. More complete

descriptions of these and other assays that can be used to test for potential genotoxicity are provided in Honeycutt, Jarvis, and McFarland (1995a,b,c).

Sediments that are to be screened are extracted and prepared as for gas chromatography/mass spectrometry analysis. Cultured cells are dosed with the sediment extracts and are then incubated for an appropriate length of time. After incubation, the cells are assayed. The assays use two types of cultured cells, H4IIE cells and Chinese hamster ovary (CHO) cells. H4IIE is an "immortal" or continuous rat liver hepatoma cell line that contains cytochrome P450. The CHO cell line is a continuous cell line that does not contain cytochrome P450. This distinction is important because many invertebrate aquatic species do not possess well-developed cytochrome P450 systems. Thus, using both cell types gives a better indication of risk to all aquatic species than does using only one type. Also, because some chemicals, such as the PAHs, must be metabolically activated in order to exert their genotoxic effect, the use of both types of cell lines can discriminate the presence or absence of these chemicals.

In Vivo Testing

In vitro testing serves to identify potentially genotoxic dredged material but does not yield information concerning bioavailability of the contaminants in the sediments. Though methods are continually being refined to predict contaminant levels in aquatic organisms (McFarland and others 1996), the genotoxic potential of dredged material must be evaluated for individual sediments on a case-by-case basis. For this purpose, *in vivo* assays will be developed to test those dredged sediments for which *in vitro* testing indicates a genotoxic potential.

Several ways to accomplish this appear to be possible. Rapidly developing larval fish (which are therefore susceptible) can be exposed to dredged material and observed for developmental abnormalities. Another possibility is development of a transgenic fish that will signal the occurrence of mutations by expression of a detectable gene product, such as firefly luciferase. A third possibility is the use of a susceptible standard fish model, such as the Japanese medaka. Exposures would necessarily be of partial lifetime duration (2 to 3 months). At the end of the exposure, the fish would be subjected to a battery of biochemical assays much like the *in vitro* screening assays. This would involve testing blood samples for alanine aminotransferase, which is indicative of cytotoxicity. Livers can be excised and analyzed for cytochrome P450 levels, DNA damage, glutathione levels, ornithine decarboxylase activity, and oxidative damage. The results of these tests can then be compared to a matrix of the effects of known carcinogenic compounds. Matrix comparisons enable interpretation of the biomarker data in terms of the effects of model genotoxic chemicals having known modes of action.

Research Efforts

The Aquatic Contaminants Team at the U.S. Army Engineer Waterways Experiment Station is currently developing and validating the *in vitro* assays that have been described in this technical note. To keep the assays rapid, inexpensive, and sensitive, multiwell fluorescence plate reader technology is being used as the basic developmental methodology whenever possible. For example, in the cytotoxicity test, H4IIE or CHO cells are plated in 96-well plates and incubated overnight. The cells are then spiked with sample extracts or chemical standards and incubated an additional 24 hr. At the end of the 24-hr exposure period, the culture medium is removed from the wells using a microplate washer. Buffer containing calcein AM is added to the cells. Calcein AM is absorbed by live cells, fluorescing green at 530 nm. The cells are read in the fluorescence plate reader, and cytotoxicity is expressed as percent viability. Similar techniques are being applied to most of the other assays in the *in vitro* genotoxicity testing battery. The development of *in vivo* genotoxicity testing methods has not yet begun.

Regulatory practices are increasingly being framed in the context of risk assessment. The assessment of risk from environmental chemicals cannot be done accurately based on acute toxic responses alone. Procedures to evaluate the effects of long-term chronic exposures on growth and reproduction in whole organisms and to extrapolate such effects to populations are still in development. Even when such tests are available, their utility will be limited by high cost and diminishing resources for regulatory implementation. In addition, many of the contaminants in sediments are genotoxic and may not be detected by chronic laboratory exposures. Risk assessments that do not include the potential for genotoxic effects when that potential exists are inaccurate. The work described here is intended to address the need for less costly and more mechanistically interpretable ways to provide the basic data on which accurate risk assessments can be conducted.

References

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INTERNET DOCUMENT INFORMATION FORM

A . Report Title: ASSESSMENT OF THE GENOTOXIC POTENTIAL OF DREDGED MATERIAL

B. DATE Report Downloaded From the Internet: 08/15/00

C. Report's Point of Contact: (Name, Organization, Address, Office Symbol, & Ph #): **US Army Engineer Waterways Experiment Station**
Environmental Laboratory
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D. Currently Applicable Classification Level: Unclassified

E. Distribution Statement A: Approved for Public Release

F. The foregoing information was compiled and provided by:
DTIC-OCA, Initials: __LF__ Preparation Date 08/21/00

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