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Proceedings of the Workshop on IN VITRO Assessment of Contaminated Sediments for Potential Carcinogenicity, January 17-19, 1989, Duluth, Minnesota

Great Lakes Water Quality Board

United States. Environmental Protection Agency

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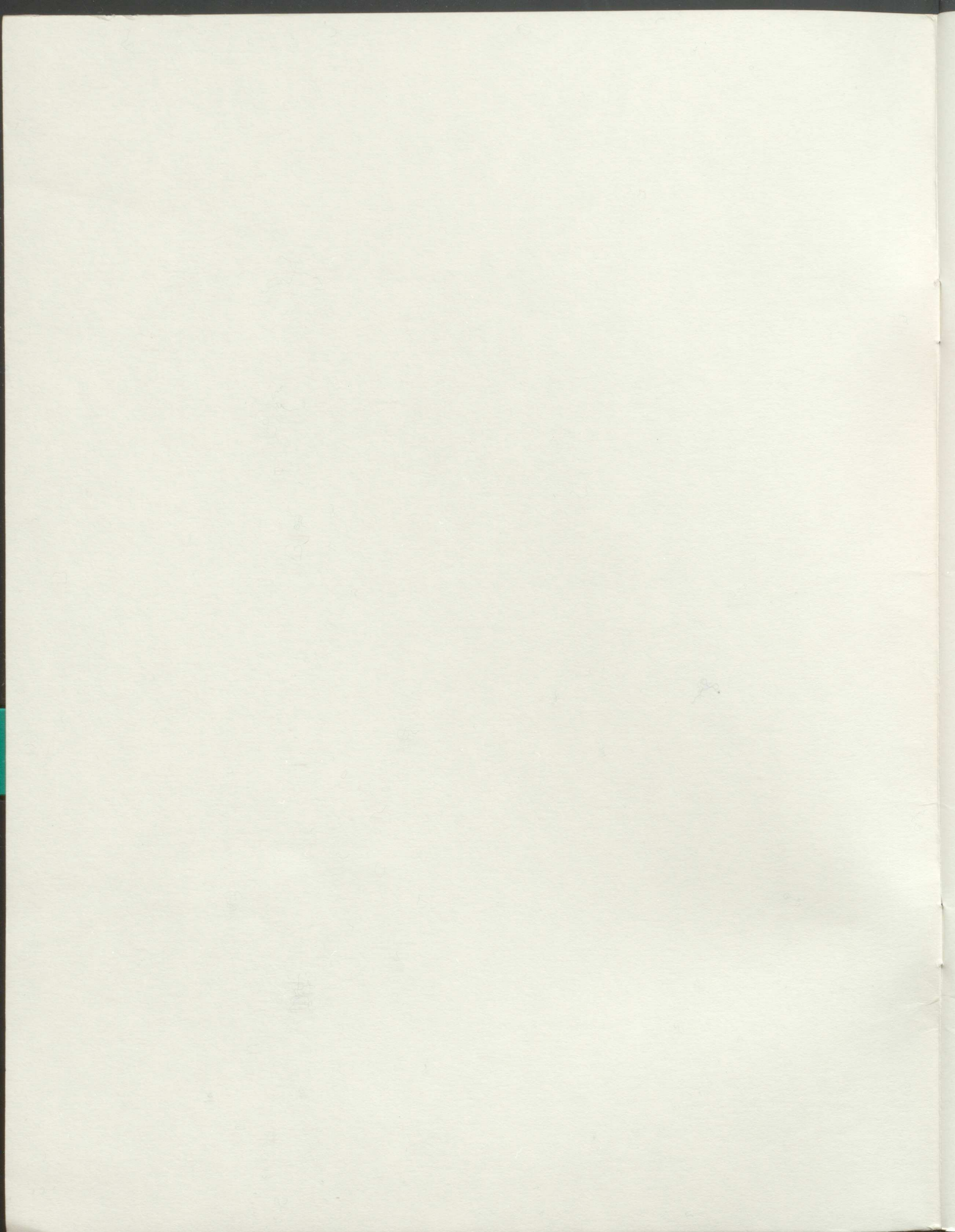
Report to the Sediment Subcommittee
of the Great Lakes Water Quality Board

Proceedings of the Workshop on
IN VITRO Assessment of Contaminated
Sediments for Potential Carcinogenicity

Proceedings of the Workshop on
IN VITRO Assessment of Contaminated
Sediments for Potential Carcinogenicity

January 17-19, 1989
Duluth, Minnesota

Sponsored by the
Great Lakes Water Quality Board of the
International Joint Commission and by the
U.S. Environmental Protection Agency

Cochairs: Michael J. Mac
Rodney D. Johnson

The views expressed in this report are those of the Workshop participants and are not necessarily those of the Great Lakes Water Quality Board or the International Joint Commission.

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Observations of cancer in wild fish populations, both in the Great Lakes and elsewhere, suggest that environmental contaminants play a critical role in the etiology of this disease. Bottom-dwelling fish have been shown to be most often affected by tumors and this may be because of their association with contaminated sediments. Sediments serve as reservoirs for a number of carcinogenic chemicals which may be available to fish through respiration, ingestion, or physical contact. In light of this, the need exists for regulatory testing of bottom sediments for their potential carcinogenicity.

Testing of sediments for carcinogenicity will be incorporated as part of the overall sediment testing strategy (Figure 1) recommended by the Sediment Subcommittee of the Great Lakes Water Quality Board (IJC 1989). Within this testing strategy, the following recommendation was made regarding the testing of sediments for carcinogenicity/mutagenicity.

"A reliable short-term test for measuring mutagenic potential of sediments is not currently available. However, until a satisfactory method is developed, existing short-term tests, such as the Ames Salmonella/microsome assay, should be performed. The results of these tests need to be used with caution because of the frequency of false negatives and the uncertainty associated with chemical mixtures. Comparison of bulk chemistry analyses with existing carcinogenicity data bases will provide supportive information."

In an effort to better understand and utilize in vitro assays available for assessing carcinogenicity, a workshop was held at the U.S. Environmental Protection Agency-Environmental Research Laboratory in Duluth, Minnesota, January 17-19, 1989. This workshop was attended by 15 invited scientists from both the United States and Canada with expertise in cancer, in vitro testing, and sediment toxicity and chemistry. The format of this workshop included formal presentations, general discussions and the development of a set of conclusions, a testing strategy, and research recommendations regarding potential sediment carcinogenicity. This document summarizes the proceedings of the workshop and makes recommendations concerning sediment testing for carcinogenic potential. It should be understood that these recommendations are made using available scientific information and that as our knowledge in this area expands, it should result in updating or altering this testing strategy.

SUMMARY OF PRESENTATIONS

This section briefly summarizes the formal presentations made during the workshop. Extended abstracts of these presentations are contained in Appendix A and should be referred to for more information.

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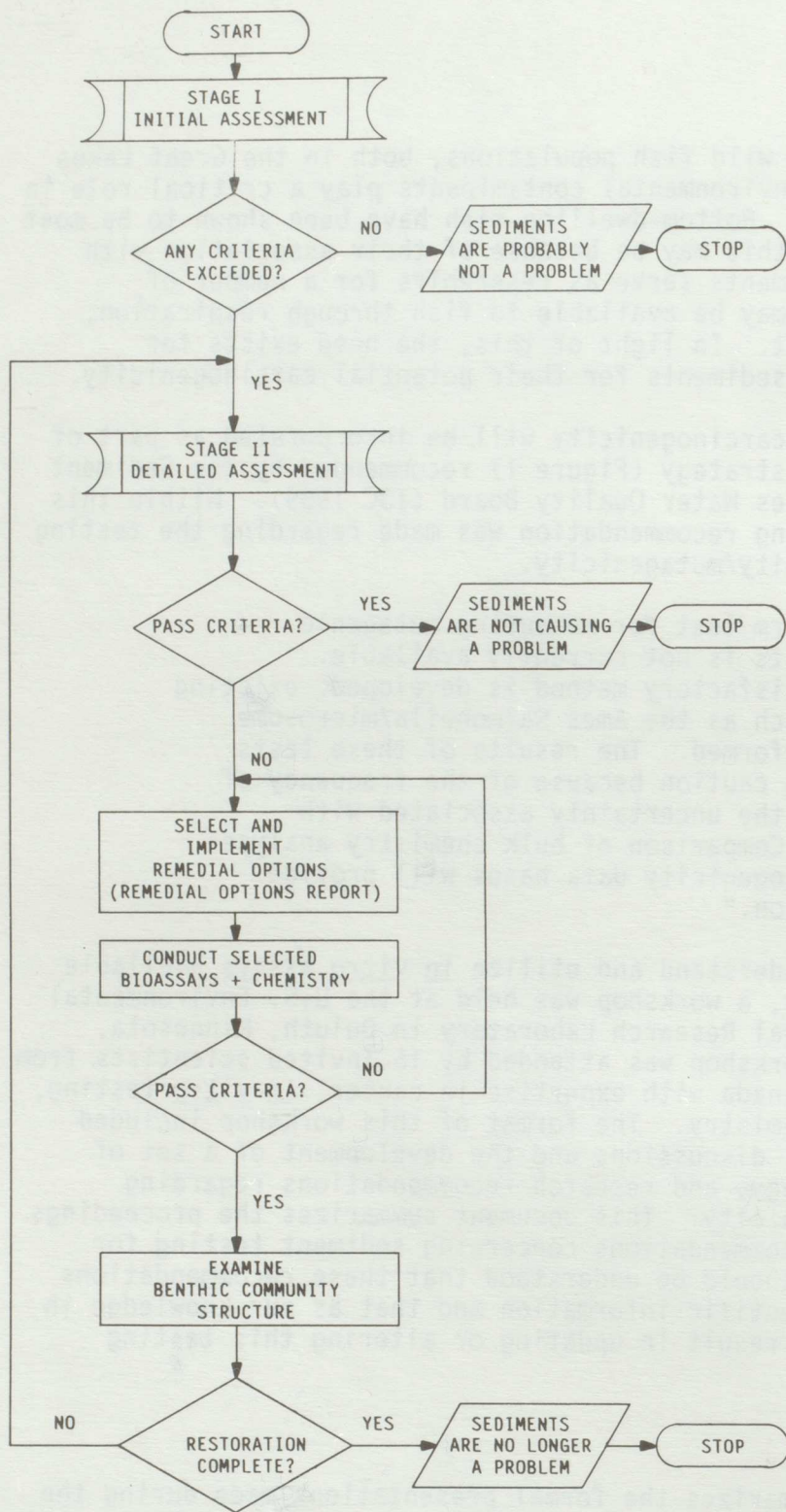
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│ STAGE I: INITIAL ASSESSMENT   │
│   │
│ Physical Description + Bulk Chemistry   │
│ External Abnormalities   │
│   (fish)   │
│ Tissue Concentrations   │
│   (adult fish & benthic invertebrates)   │
│ Community Structure   │
│   (benthic invertebrates-family level)   │
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│ STAGE II: DETAILED ASSESSMENT   │
│   │
│   PHASE 1   │
│   │
│ Physical Mapping   │
│   │
│   PHASE 2   │
│   │
│ Surficial Chemistry   │
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│ Algal Photosynthesis   │
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│ Ames   │
│ Cores   │
│ Fish Tumors   │
│   │
│   PHASE 4   │
│   │
│ Sediment Dynamics   │
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*(AFTER IJC 1989)

Dr. Michael Mac presented the introduction and a set of objectives to the workshop. The introduction included background information on the need for sediment assessment, a brief history of sediment contamination problems, and the Sediment Subcommittee's recommended protocol. A set of four objectives for the workshop were proposed:

a)   Evaluate in vitro methods for predicting potential carcinogenicity of sediment associated compounds;

b)   Specifically examine the use of the Ames Salmonella/microsome assay (hereinafter referred to as the Ames test) with various sediment extracts;

c)   Establish a protocol for testing sediment carcinogenicity using in vitro methods if appropriate;

d)   Recommend areas of research and development that would improve our predictive capability regarding sediment carcinogenicity;

Dr. Paul Baumann presented data on liver cancer incidence in feral fish populations and evidence that sediments contaminated with polynuclear aromatic hydrocarbons (PAH) correlated well with the occurrence of tumors. It was also pointed out that limited sampling of sediments and their spatial heterogeneity as well as uncertainty of fish movements prevented establishing clear cause and effect relationships in the field.

Dr. Spalding discussed the relationships between the results of short term in vitro assays and rodent carcinogenicity bioassays. The mutagenicity assays showed good agreement among one another and were effective at identifying genotoxic carcinogens. However, he concluded that mutagenicity assays (such as the Ames test) do not provide information applicable to the evaluation of nongenotoxic carcinogens which constitute 35-40% of known rodent carcinogens.

Dr. David Hinton identified fish species that showed promise for carcinogenicity testing, and listed compounds that produce tumors in fish. He also reviewed exposure routes and latency periods from fish cancer studies and identified tissues shown to be capable of developing tumors.

Dr. Russell Malcolm presented results of studies that demonstrate induction of tumors in fish through exposure to contaminated sediments and/or diet. In vitro tests (including Ames) conducted on extracts of these sediments indicated that certain fractions were mutagenic.

Dr. David Rokosh reviewed the literature on mutagenicity testing of environmental samples. Several factors such as unknown chemical composition, interactions in chemical mixtures, potential direct toxicity to test animals, and varying the amount of S-9 used for metabolic activation complicate interpretation of in vitro mutagenicity assay results.

Dr. Alex Maccubbin reviewed physiological responses of fish to carcinogenic chemical exposure. Knowledge of fish metabolism and immune systems is highly deficient.

Mr. Michael Fox spoke on chemical extraction and fractionation of sediments. One fractionation protocol was recommended that comprises a number of established extraction methods. The advantage of fractionating samples is that it permits the identification of specific mutagens and a reduction in toxicity to bacteria compared with crude extracts. Fractionation can also allow the detection of responses that may be masked by the presence of inhibiting chemicals. However, extensive fractionation will create additional samples for testing.

CONSENSUS CONCLUSIONS

1. A cause-effect relationship is strongly indicated between contaminated sediments and neoplastic disease in fish. The clearest demonstration is for liver (and possibly skin) neoplasia in fish exposed to sediments contaminated with polynuclear aromatic hydrocarbons (PAHs). Several studies supply the following evidence to support this conclusion.

   o   the presence of mutagens and carcinogens in sediment and tissue residues

   o   trophic transfer of potential carcinogens from invertebrates to fish

   o   the presence of enzyme pathways in liver and other organs for the metabolism of promutagens and procarcinogens into their ultimate mutagenic and carcinogenic forms

   o   the formation of DNA adducts in liver cells of fish exposed to polluted sediments

   o   the occurrence of liver and sometimes skin tumor epizootics in 14 species of wild fish from over 35 locations where chemicals are concentrated

   o   the experimental induction of liver and skin neoplasms in fish and mice with extracts of sediment from areas where epizootic liver neoplasms occur in wild fish

   o   the experimental induction of liver cancer plus cancer at other tissue sites with approximately three dozen known carcinogenic chemicals in one or more of numerous species of fish

2. Fish can be exposed to sediment-associated, cancer-causing chemicals through several routes. Uptake may occur through trophic transfer from benthic invertebrates. Alternatively, the equilibrium partitioning of these chemicals between the sediment and water permits the direct absorption of these agents via the gills.

3. Fish are good indicators of environmental quality, through either their absence in otherwise suitable habitat, or through the presence of tissue lesions including neoplasia. Moreover, fish are good sentinels of possible effects in humans because toxicological effects found in one phylum are likely to be relevant indicators of effects in other phyla. In other words, toxicological effects in lower vertebrates are relevant to humans. Since chemicals in sediments have caused adverse effects on fish,

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direct or indirect exposure of humans to sediment contaminants poses a risk to human health. Possible exposure routes to human include direct dermal contact and ingestion of contaminated food and water.

4. Reliable in vitro assays exist that can be used to detect genotoxic chemicals. In short-term tests, demonstrated genotoxicity indicates the potential to induce heritable genetic effects in offspring, as well as cancer in exposed organisms. Because mutation is an endpoint that can indicate direct impact on health, it should be regarded as an adverse toxicological effect per se. Accordingly, it is prudent to consider sediments that test positive in the Ames test as a potential hazard to biota.

5. Environmental carcinogens can be classified as either genotoxic or nongenotoxic. Standardized and validated in vitro assays exist which can be used to detect genotoxic chemicals, e.g. the Ames test can detect genotoxic carcinogens with a high degree of probability (Tennant et al. 1987). Validated in vitro assays for detecting nongenotoxic carcinogens are not currently available, but are under development. Because of the significant proportion of carcinogens that may be nongenotoxic (40%, Ashby and Tennant, 1988) a valid bioassay for their detection is urgently needed and should be incorporated into a sediment assessment protocol when available.

6. The Ames test is recommended for the first tier screening of sediments for genotoxic chemicals. Positive results confirm the presence of mutagenic compounds and suggest that additional testing is required to define the hazard associated with exposure to the sediment. Since inherited genetic disease and carcinogenesis are in vivo processes, in vitro tests are not direct measures of carcinogenesis. Therefore, to verify or increase the confidence in any hazard assessment based upon in vitro responses, an in vivo bioassay is strongly recommended and is essential where human exposure is expected.

7. A negative result in the Ames test is not a reliable, stand-alone indication that sediments are not carcinogenic. The Ames test does not detect nongenotoxic carcinogens and does not detect all genotoxic carcinogens; therefore, a second-tier confirmation of a negative Ames test result is required under virtually all circumstances.

8. The in vitro assessment of sediment associated contaminants should be made initially using chemical extraction procedures, thereby testing the major chemical fractions present. Pore water and elutriates do not adequately represent all potentially bioavailable chemicals in contaminated sediments.

ASSESSMENT STRATEGY

Within the framework of the sediment evaluation protocol established by the Sediment Subcommittee, information will be generated at several levels that will contribute to the identification of potential mutagenic/carcinogenic impacts of sediment. Chemical analyses of surficial sediments can be reviewed for the presence of known carcinogens using existing databases (e.g. Gene-Tox carcinogen database, Nesnow et al. 1987). In addition, the results of tumor surveys in fish may indicate the presence of carcinogenic contaminants in the

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sediments. For each site, decision-making should be based on field surveys as well as on results generated by mutagenicity assays.

MUTAGENICITY ASSESSMENT

The following guidelines should be followed to standardize the assessment process and to maximize inter-site comparisons.

1. Of the in vitro tests available, the Ames test should be used first to assess mutagenicity of a sediment.

   a. If this test is positive, then the sediment should be considered genotoxic.

   b. If this test is negative, then at least one additional in vitro test should be applied. The second in vitro test should be a chromosome aberration assay that uses mammalian cells, e.g. Chinese hamster ovary cells (Ashby 1986). Use of a second in vitro test is recommended since the Ames test is not effective at detecting all genotoxins. If the second in vitro test is positive, then the sediment should be considered genotoxic. If the second in vitro test is negative, then the sediment should not be considered genotoxic.

2. When a sediment is determined to be genotoxic, a review of possible routes and extent of human exposure should be investigated. If significant human exposure appears likely, then in vivo testing is recommended.

3. When a sediment is determined not to be genotoxic using the recommended testing scheme, no conclusions can be made regarding the potential nongenotoxic carcinogenic hazard. Additional testing should be considered if tumors are found in a survey of feral fish in the area or if analytical chemistry of surficial sediments indicates the presence of significant amounts of suspected or known carcinogens.

4. When possible, the initial Ames test should be done on crude extracts of the sediment as indicated in conclusion number eight. However, toxicity in assays of crude extract may preclude the successful assessment of mutagenicity. In this case, fractionation of the sediment extract may be required. Existing analytical information as well as current and historical input data should be used in the design of the fractionation protocol. Considerations in the development of a fractionation scheme include:

   a. extraction by a graded series of nonpolar and polar solvents;

   b. adjusting pH to promote the release of acidic and basic organic chemical residues and metals;

   c. potential alteration of the form of chemical contaminants in the sample due to extraction solvents and methodology;

d. compatibility of chemicals used in fractionation with the
     biological assessment techniques.

  A site-specific extraction and fractionation procedure should be
  developed where needed. If the intent is to identify potential
  genotoxic compounds, then fractionation will be necessary.

INTERLABORATORY CALIBRATION

There are numerous sources of variation in most laboratory investigations
including in vitro mutagenicity assays. This variation may lead to systematic
differences between results from different laboratories or within the same
laboratory when tests are performed at different times. It is important to
limit this variation which may arise due to differences in sediment
composition laboratories, specific techniques and other factors. Validation
of in vitro tests should be used when tests are performed on sediment
extracts. The International Joint Commission should organize a round robin
assessment of interlaboratory and intralaboratory variation to support any
program for in vitro sediment assays. To facilitate this calibration
endeavor, a standardized sediment should be provided. A clear definition of
how to prepare or obtain a standardized S-9 activation fraction needs to be
included in the round robin tests, and ultimately in the overall application
of in vitro tests requiring microsome activation.

AMES PROTOCOL

Several existing protocols for the Ames test are available for
mutagenicity testing of single chemicals. These can be adapted for sediment
(complex mixture) testing (Maron and Ames, 1983; Claxton et al. 1987).
Genotoxicity testing of complex mixtures from matrices other than sediments
has also been done (Lewtas 1988, Andon et al. 1986; Austin et al. 1985).

The application of these assays to the problems of screening sediment
samples for mutagenicity requires the consideration of many factors including
interactions due to the presence of multiple chemicals, cost-effectiveness of
the assay and expected use of the assay results. To assist in the development
of and use of the Ames test results for the purpose of sediment screening and
classification, we make the following recommendations.

Salmonella Strains

It has been demonstrated that two tester strains (TA98 and TA100) are
capable of detecting the majority of genotoxic carcinogens in the NTP data
base. Therefore, routine screening of sediment samples should use, at a
minimum, tester strains TA98 and TA100. In specific instances, other tester
strains sensitive to certain classes of compounds should be considered when
these chemicals are known to be present in sediment samples (based on
available knowledge of local industries, residue analysis, etc.). Thus the
probability of detecting these classes of chemicals can be maximized. Because
of cost-benefit considerations, we do not recommend the routine use of strains
other than TA98 and TA100.

Reproducibility

All _in vitro_ tests should be repeated and experimental parameters altered as necessary to maximize the potential for confirming a positive or negative result. Three replicate plates should be scored for each dose level of the test sample and for the negative and positive controls. Each test sample (fraction) should be tested in the absence and presence of an exogenous metabolic activation (S-9) component. If multiple samples collected from the same geographic locations are tested, then repetition is inherent in the analysis of multiple samples. In this instance, repeated testing of all samples would not be cost-effective. However, prior to testing the multiple samples, range finding studies should be conducted on representative samples to ensure that experimental conditions have been optimized.

Exogenous Metabolic Activation Components

All sediment samples should be tested both with and without Aroclor 1254 induced rat liver S-9 fractions. The S-9 preparations should be standardized and the use of multiple S-9 concentrations is recommended. Although alternative sources of S-9 preparations (e.g. fish) may improve the correlation of _in vitro_ mutagenesis results with the observations of tumor incidence in fish surveys, their use would be largely experimental and exposure to sediment associated chemicals is not limited to fish populations.

Controls

An evaluation of appropriate negative and positive control substances should be performed in every test to serve as indicators of test performance. In addition to the controls recommended for existing standard protocols (Maron and Ames, 1983), we recommend using several additional. Potential controls include a) tests to determine the positive or negative effects of solvent extraction and fractionation schemes; and, b) tests to evaluate the possibility that overt toxicity may mask the expression of mutagenicity; (e.g. the incorporation of known mutagens into the sediment fractions).

Statistical Analysis

Several methods are available for the statistical analysis of Ames test results (Margolin et al. 1981). In general, a two-fold increase in revertants together with evidence of a dose-dependent response are sufficient to conclude that the test sample exhibits mutagenic activity. The relative potencies of sample activity (e.g. the ranking of the relative potencies of samples from different sites) may require analysis by risk assessment models.

RESEARCH RECOMMENDATIONS

1. **Storage of Sediment Samples:** The storage of sediments or sediment extracts can change the chemical characteristics and measured biological effects of the sample.

   Investigations are needed to determine optimal storage practices for both whole sediment and sediment extracts.

2. **Sample Extraction, Concentration and Fractionation Methods:** Various methods have been used to extract, concentrate and fractionate contaminants from sediment samples. Most of these methods have been developed to optimize conditions for subsequent chemical analyses.

    Available extraction, concentration and fractionation methods should be evaluated to find optimal conditions for use in in vitro and in vivo assays.

3. **Sample Compositing:** Compositing of sediment samples may be a useful strategy for site survey investigations because it may be cost-effective to conduct extensive chemical and biological testing on a limited number of composited samples representing an area. After composite sample evaluation, less extensive chemical/biological testing of individual samples might be sufficient to characterize the extent of sediment contamination.

    Research should be done to study the chemical dilution effects that may result from sediment sample compositing.

4. **Activation Metabolism in Fish:**

    The metabolic activation of carcinogens by fish needs to be investigated further. This work could establish links between neoplastic disease in feral fish and sediment contaminants.

5. **Nongenotoxic Carcinogen Assays:**

    Appropriate agencies in the United States and Canada should vigorously pursue the development and validation of in vitro assays for nongenotoxic carcinogens.

6. **Fish Carcinogenesis Assay:**

    Appropriate agencies in the United States and Canada should pursue the development and validation of carcinogenesis assay systems using small aquarium fish (e.g. Japanese medaka) for use with individual chemicals and complex mixtures (e.g. contaminated sediments). Such a system should include biochemistry/metabolism and pharmacokinetics as well as cancer pathology.

7. **Fish Neoplastic Disease Epizootics:** Harshbarger and Clark (1989) recently summarized neoplasm incidence reports, and further work of this type is essential.

    Reports of epizootics and neoplastic diseases in fish, and their relationship to chemical exposure should be evaluated thoroughly using epidemiological study criteria (e.g. Sindermann 1979; Susser 1986).

8. **Wildlife Neoplastic Disease:** There are very few reports of neoplastic disease in wild birds and mammals that may have been exposed to chemical carcinogens in the Great Lakes region or elsewhere. These kinds of reports are potentially important in linking neoplastic disease in fish to cancer risk in higher vertebrates.

Autopsy reports at wildlife research centers need to be reviewed and
populations of animals from the field that have a high probability of
exposure (e.g. harbor rats) need to be sampled.

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APPENDIX I

EXTENDED ABSTRACTS

ASSOCIATION OF CARCINOGENS IN SEDIMENT WITH FISH NEOPLASIA

by

Paul C. Baumann
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Epizootics of liver neoplasms have been reported in benthic feeding fishes from a variety of marine and freshwater locations. Most of these locations have sediments contaminated by industrial pollutants. Many, such as the Black and Cuyahoga Rivers, Ohio, the Buffalo River, New York; and numerous locations in Puget Sound, are characterized by elevated levels of Polynuclear Aromatic Hydrocarbons (PAHs). Liver cancer occurred in 20% to 40% of mature brown bullhead from the Black and Cuyahoga rivers in the early to mid 1980s. PAH sediment levels in these rivers was in the part-per-million to tens of parts-per-million (ppm) range for individual compounds including carcinogens such as benzo(a)pyrene (B(a)P). Six locations having high frequencies of liver neoplasia in fish (three locations in Puget Sound and three Lake Erie tributaries) had a range of PAH concentrations one hundred times higher than did three freshwater and two marine reference locations. Therefore, in general, epizootics of liver tumors in wild populations of fish have been associated with high concentrations of PAHs in sediment. Chlorinated compounds, however, including dioxins and dibenzofurans, were highest in the Fox River, Wisconsin, where brown bullheads were found not to have liver tumors.

Although PAH concentrations in sediment and tumor epizootics in fish seem to be positively correlated in general, the use of PAH sediment values to predict tumor frequencies in benthic fishes is not currently feasible. This has been demonstrated for Puget Sound, where tumor frequencies in English sole, bile metabolites fluorescing at B(a)P wavelengths, and sediment PAH concentrations were all determined for a variety of locations. Although metabolite levels and tumor frequencies were well correlated, the same could not be said of PAH sediment values. This lack of correlation between sediment PAH levels and bile metabolite concentrations points up the dual problems of patchy environmental distribution of the parent compounds (causing sampling problems) and the mobility of fish. In Elliot Bay, Puget Sound, for instance, both fluoranthene and B(a)P concentrations in sediment may vary by two to three orders of magnitude among locations.

An additional problem in comparing locations is that the composition of the PAH mixture may vary greatly. Even within the Buffalo River, New York, for example, the highest concentrations of fluoranthene and of B(a)P occur at different sampling sites, and their distribution patterns within the river are different. Such differences in the relative abundance of specific compounds within a mixture of PAHs is usually exaggerated when comparing different systems having different types of point sources. The carcinogenicity of such mixtures cannot be estimated with current knowledge. However, it is not simply the additive value of the individual carcinogens present. Dr. John J. Black in experiments with Swiss mice proved that an extract from the Black River, Ohio was more carcinogenic than a positive control of pure B(a)P, even though the additive concentrations of carcinogens in the extract were much less than the B(a)P dosage used for the control. Thus, the carcinogenicity of

the Black River extract was due to transport effect, promotional effects,
synergistic effects, or a combination of these. Therefore, until more
experimental information is obtained on the interaction of PAHs within a
mixture in biological systems, a general correlation between high PAH in
sediment and elevated tumor frequencies in benthic fishes is all that can be
expected.

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COLLECTION, EXTRACTION AND ANALYSIS OF SEDIMENT AND
PORE WATER SAMPLES: SIGNIFICANCE TO SEDIMENT TOXICITY TESTING

by

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A large body of published data on organic contaminants in surficial
sediments is available. The most widely used collection, storage and
analytical procedures are discussed and their significance to toxicity testing
considered.

SAMPLE COLLECTION

Typically, a grab sample up to 10 cm deep is used. This is not a very
realistic interface sample. The top section of a "Benthos" type sediment core
is better.

PRESTORAGE TREATMENT

Inert debris such as stones and shells should be removed. Macrobenthos
such as amphipods should also be removed.

STORAGE

Sediment samples are frequently freeze dried. Some loss of volatile
components may occur. Samples should be immediately frozen and stored frozen
prior to a wet extraction technique to avoid volatile losses.

EXTRACTION METHODS

Soxhlet extraction and sonication are most often reported. Soxhlet
extraction for up to 24 hours is favoured by a majority of laboratories.

EXTRACTION SOLVENTS

Single solvents such as acetonitrile or dichloromethane are reported.
More users favour polar/nonpolar solvent mixtures such as acetone/hexane.
This may be followed with a second extraction with a very polar solvent such
as methanol. The range of compounds extracted is made more complete by
performing extractions at both high (11) and low (2) pH values.

CLEANUP AND FRACTIONATION

Lipids and high molecular weight material of plant and animal origin may
be removed by gel permeation chromatography. The concentrated extracts are
eluted through silica gel or Florisil columns with solvent mixtures of
increasing polarity. Typical eluants are hexane followed by 20%
dichloromethane in hexane and finally dichloromethane. The individual
fractions may then be tested for mutagenic potential. An extraction and
cleanup procedure yielding up to eight distinct fractions improves the chances

of relating mutagenic response to specific compounds and reduces the masking
of mutagenic response of one compound by the acute toxicity of others.

ANALYSIS OF EXTRACT FRACTIONS

The nonpolar and slightly polar fractions are usually analyzed by
capillary gas chromatography with a variety of detectors. The mass
spectrometry detector (GC-MS) operated in the electron impact mode yields
useful structural information on unknown compounds. The electron capture
detector (GC-EC) and the mass spectrometer operated in the negative chemical
ionization mode (GC-MS-NCI) give a more sensitive response to electrophiles
(most carcinogens) but little or no structural information and are best suited
for confirming the presence of specific compounds. The polar fractions may be
analyzed by high performance liquid chromatography (HPLC) using ultra-violet
or fluorescence detectors.

PORE WATER

Very little published data on organic contaminants in pore water exist.
This is largely due to the difficulty of analyzing the typically small samples
from the recovery methods available. Methods for collecting pore water
include centrifugation, compression, osmotic diffusion and
suction/filtration. All yield very small (<10 mL) volumes and some may
rupture living cell walls and contaminate the sample. Colloidal organic
carbon in the pore water is also believed to reduce the bioavailability of
"dissolved" compounds. Hypothetical animal - water equilibrium relationships
for oligochaetes and amphipods (Bierman 1987) suggest that the true aqueous
concentrations in the interstitial water are not substantially different than
aqueous concentrations in the water column. Connor (1984) has produced an
expression for animal:sediment concentration ratios which contain no water
term. Thus, the requirement for pore water concentrations can be avoided.

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PRODUCTION OF TUMORS IN FISH FOLLOWING LABORATORY
EXPOSURES TO CARCINOGENS

by

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A variety of compounds have been tested in fish species and proven to cause development of neoplasia in a variety of organs/tissues. Work begun in the late 1950s in relation to an outbreak of hepatomas in trout fed diet stored under less than optimal conditions (Halver and Mitchell, 1967) has now progressed to use of small aquarium fishes and to exposure at various life stages (Hoover et al. 1984).

Table 1 presents a partial listing of compounds proven carcinogenic in fish bioassay. Compounds include: aromatic amines, azo compounds, halogenated organic compounds, mycotoxins, n-nitroso compounds, plant derivatives, polynuclear aromatic compounds and various miscellaneous compounds.

Both freshwater and marine/estuarine species have shown promise for in vivo carcinogenesis testing. A partial listing of these species is given in Table 2. Freshwater indigenous species include: rainbow trout (Oncorhynchus mykiss), brown bullhead catfish (Ictalurus nebulosus) and topminnows (Poeciliopsis species). Trout, specifically the Mt. Shasta Strain, have proven very sensitive to certain genotoxic carcinogens and a large data base exists for this species (Hendricks 1982; Hoover et al. 1984). Brown bullhead catfish, in field studies, have proven susceptible to tumorigenesis in the liver. The use of this species in laboratory exposures, however, has received only limited attention. The topminnows have proven sensitive to the PAH, 7,12 dimethylbenz(a)anthracene.

Aquarium species, not native to the U.S., have received extensive application, and one of these - the Japanese Medaka (Oryzias latipes) - is emerging as a top candidate for continued use and expansion as a model species. Other exotic species, guppy (Poecilia reticulata) and the platyfish hybrids (Xiphophorus species) have important roles in fish carcinogenesis studies.

In general, fewer species of marine/estuarine fishes have been used in trial exposures. The sheephead minnow (Cyprinodon variegatus) is sensitive to the tumor causing potential of diethylnitrosamine. The lesions in liver have been compared with those seen in similarly-exposed rodent species (Couch and Courtney, 1987). The truly hermaphroditic species, rivulus (Rivulus marmoratus), a native of Caribbean tidal pools, is sensitive to diethylnitrosamine. Offspring from a single parent may be used to eliminate potentially confounding genetic variability associated with other species. Two flatfish species, winter flounder (Pseudopleuronectes americanus) and English sole (Parophrys vetulus), develop tumors in the wild. However, the effective use of these as laboratory animal models has yet to be realized.

TABLE 1

COMPOUNDS PROVEN POSITIVE IN FISH CARCINOGENESIS BIOASSAY

Aromatic Amines
    Acetylaminofluorene

Azo Compounds
    o-Aminoazotoluene
    4-Dimethylaminoazobenzene

Halogenated Organic Compounds
    Carbon Tetrachloride
    Dichlorodiphenyltrichloroethane
    Mixtures of Chlorine Residuals

Mycotoxins
    Aflatoxin
        B_1
        Aflatoxicol
        G_1
        M_1
        O_1
        Versicolorin A
        Sterigmatocystin

N-Nitroso Compounds
    N-Nitrosodiethylamine
    N-Nitrosodimethylamine
    N-N'Dinitrosopiperazine
    Nitrosomorpholine
    N-Methyl-N-Nitroso Urea
    N-Ethyl-N-Nitroso Urea
    N-Methyl-N-Nitro-N-Nitroso Guanidine

Plant Derivatives
    Cyclopropenoid Fatty Acids
    Cycasin
    Methyl Azoxymethanol Acetate

Polynuclear Aromatic Compounds
    Benzo-a-Pyrene
    7, 12 Dimethylbenz(a)anthracene
    3-Methylcholanthrene

Miscellaneous Compounds
    Nifurpirinol
    Thiourea
    Urethane

TABLE 2

PROMISING FISH SPECIES FOR CARCINOGENESIS TESTING

FRESHWATER

Indigenous

Rainbow Trout (Oncorhynchus mykiss)

Brown Bullhead Catfish (Ictalurus nebulosus)

Topminnows (Poeciliopsis spp)

Exotic (Laboratory)

Guppy (Poeciliae reticulata)

Platyfish Hybrids (Xiphophorus species)

Japanese Medaka (Oryzias latipes)

MARINE/ESTUARINE

Rivulus (Rivulus marmoratus)

Sheepshead Minnow (Cyprinodon variegatus)

Winter Flounder (Pseudopleuronectes americanus)

English Sole (Parophrys vetulus)

From original restriction to adults in bath exposures, carcinogen bioassay now employs a variety of life stages and exposure routes, listed in Table 3. Embryo fish are exposed by direct microinjection of test compound into the yolk sac or perivitelline space. Alternatively, swollen eggs are exposed using direct topical application of test compound in carrier vehicle. Hatchlings are also exposed via microinjection (sac fry-trout) or via bath exposure of high concentration and short duration. When adults are used, dietary, bath, injection or skin painting are routes of exposure.

One _inherent_ advantage associated with small fish species in carcinogen testing is the brief time required for tumor production. Depending upon the age, species of fish and the compound used, latency periods of from 8 to 40 weeks have been reported. Japanese medaka have very short durations between exposure and endpoint.

If tumors from feral fish and laboratory fish are considered together, tumors have been reported in each organ of fish. However, laboratory trials primarily cause tumors of the liver. Kidney, intestine, skin, swim bladder, eye and pancreas are other target organs (Table 4).

Based on brief latency periods, sensitivity, cost and reduced space limitations, the use of small aquarium species and early life stages of larger fish is recommended in testing sediment and their extracts for cancer-causing potential.

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TABLE 3

METHODS OF EXPOSURE BY LIFE STAGE

EMBRYO

    Bath Using Short Pulse and High Concentration
    Microinjection

HATCHLING

    Sac Fry - Microinjection
    Bath - High Concentration, Short Duration

ADULTS (OR SEXUALLY IMMATURE SUBADULTS)

    Dietary
    Bath
    Injection
    Skin Painting

TABLE 4

TUMOR SITES

             Liver
             Kidney
             Swim Bladder
             Intestine
             Eye
             Pancreas (Exocrine)
             Skin

APPLICATION OF IN VITRO TESTS IN ASSESSING MUTAGENIC POTENTIAL OF SEDIMENTS AND THE RELATIONSHIP TO FISH TUMORS. PART II

by

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The relationship between mutagenic potential in sediments and fish tumors can be examined within the framework of the multi-stage process of chemical carcinogenesis. Assuming that probable stages of carcinogenesis include initiation, promotion, progression and tumor formation, data on mutagenic potential of sediments and fish tumor incidence examine only two stages within the process. In general, in vitro assays that provide information about mutagenic potential of sediments, demonstrate the presence of potential initiators. Whether those initiators (mutagens) can cause the biological endpoint of tumor formation depends on a number of events and modulators of these events. These events include exposure to the chemical, absorption, biodistribution, metabolism, reaction with macromolecules and finally expression of biological endpoints such as toxicity, teratogenicity, mutagenicity and carcinogenicity. These events may be modified in many ways by many factors. Exposure depends upon the trophic level, habits and habitat of fish and bioavailability, persistence and physical-chemical properties of chemicals. Metabolism may be modified by species, sex, age, metabolic competence, balance between phase I and phase II enzymes and possibly extrahepatic vs. hepatic metabolism. Reaction with macromolecules depends upon the availability and number of receptors and trapping agents for reactive molecules. Finally, the expression of the effects of reactions with macromolecules can be modified by repair, growth, differentiation, promoters, cocarcinogens, gene stability, immunologic status and endocrine status of fish, as well as nutrition, diseases and possibly parasites.

There are several case histories within the Great Lakes ecosystem that document mutagenic potential in sediments and the existence of fish with tumors. These studies along with those on fish and sediments of Puget Sound, Washington provide evidence for a link between sediment-bound chemicals and neoplasia on fish. However, the predictive value of positive mutagenesis using in vitro bioassays for fish tumors still remains questionable because of a lack of knowledge about the sequence of events between exposure and a tumor endpoint and more importantly the factors that modify the process of carcinogenesis at the various stages.

IN VITRO TEST RESPONSES WITH EXTRACTS OF A TUMORIGENIC SEDIMENT: CORRELATIONS WITH NEOPLASTIC DISEASE INDUCED IN TWO SEDIMENT-EXPOSED MARINE SPECIES

by

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A battery of short-term tests (mutation in Salmonella, sister chromatid exchange in Chinese hamster V79 lung fibroblasts, and inhibition of gap-junctional intercellular communication in V79 cells) was applied to whole and fractionated solvent extracts of a chemically-contaminated marine sediment demonstrated to cause neoplastic disease in flounder and oysters. Responses in assays designed to detect genotoxic agents (mutation in Salmonella and sister chromatid exchange in V79 cells) correlated with the presence of indirect-acting, genotoxic carcinogens identified in the sediment. Without exception, exogenous metabolism was required to detect genotoxic agents, suggesting the absence of low concentration of direct-acting genotoxic carcinogens. Significant inhibition of gap-junctional intercellular communication, a possible in vitro biomarker for tumor promoters, was obtained only with F4 of the tumorigenic sediment. Although this response suggested the presence of one or more tumor promoters, known tumor promoters identified in this sediment would have been in other extract fractions. Thus, effects on gap-junctional communication were not due to effects of identified promoters. In combination, the short-term test responses correlated with the neoplastic disease induced in exposed animals.

INTRODUCTION

The findings presented here result from a recent collaborative study with the National Cancer Institute. This study was designed to evaluate the capacity of specific sediments to induce neoplastic disease in the winter flounder and American oyster. Details of this study are available (Gardner et al. 1987). A contaminated sediment from Black Rock Harbor, Bridgeport, Connecticut, was the test sediment. A relatively clean sediment from central Long Island Sound served as the reference sediment. The collaborative study was based on earlier, preliminary evidence that exposure to Black Rock Harbor sediment induced kidney and reproductive tract tumors in oysters and proliferative lesions in the pituitary gland of winter flounder. As part of the collaborative study, solvent extracts of the experimental and reference sediments were tested in selected in vitro assays designed to detect genotoxic carcinogens and tumor-promoting chemicals. The purpose of the in vitro testing was to determine (1) if the carcinogenic potential of the sediments could be characterized with such assays, and (2) to test correlations between in vitro assay responses and the presence of specific carcinogenic or

tumor-enhancing chemicals in the extracts. Summarized below are the methods used in sediment extraction and the testing of sediment extracts. In vitro test results and test organism pathology are summarized along with selected information on sediment chemicals.

METHODS

Details of all methods and testing procedures used in the study may be found in Gardner et al. 1987. The procedures used in sediment extract preparation and in vitro testing are briefly summarized below.

Sediment Extract Preparation

A protocol based on compound polarity was followed for the fractionation of sediment extracts. Sediments were extracted with acetonitrile, water added, and the extract partitioned into pentane. Pentane mixtures were brought to dryness and redissolved in dimethylsulfoxide for testing as a whole extract. Extract fractions were prepared by placing the pentane mixtures on silica gel columns. The columns were then sequentially eluted in four steps. The F1 fractions were eluted with pentane only and contained highly nonpolar compounds such as PCBs; F2 fractions, eluted with an 80:20 pentane:methylene chloride mixture, were PAH fractions; F3 fractions, eluted with methylene chloride, contained polycyclic aromatic ketones, carbazoles and phthalates; F4 fractions, eluted with methanol and containing the most polar compounds, were largely uncharacterized. For in vitro testing, each fraction was brought to dryness then redissolved in dimethylsulfoxide. Because this basic fractionation procedure was also used to prepare sediment samples for chemical analysis, in vitro responses could be evaluated in relation to specific chemical substances present in test mixtures.

In Vitro Assays

The Ames test for mutagens, sister chromatid exchange in Chinese hamster V79 cells and inhibition of gap-junctional intercellular communication in V79 cells were used to evaluate sediment extracts. The Ames Salmonella/microsomal assay with preincubation (Ames et al. 1975; Williams and Preston, 1983) was used with strains TA98, TA100, TA102 and TA104 to detect the presence of mutagenic agents. Sister chromatid exchange in Chinese hamster cells was used as a measure of genotoxicity in eucaryotic cells (Latt et al. 1981). Inhibition of gap-junctional intercellular communication was used as a possible biomarker for tumor promoters and selected nongenotoxic carcinogens (Yotti et al. 1979; Malcolm et al. 1985).

RESULTS

Pathology

Tables 1 and 2 give frequency data for selected neoplastic and other proliferative lesions identified in the American oyster (Crassostrea virginica) and winter flounder (Pseudopleuronectes americanus) exposed in the laboratory to test and reference sediments (Gardner et al. 1987). The continuous exposure of suspended test sediment for 30 and 60 days induced multiple types and formations of tumors. The highest frequency of tumor induction occurred in renal excretory tissues, followed in decreasing order by

tumors in gill, gonad, gastrointestinal, heart and neural tissues. The lack of tumor regression during 30 and 60 day post-exposure periods suggests the lesions were autonomous.

TABLE 1

PERCENT OYSTERS WITH NEOPLASTIC DISEASE FOLLOWING LABORATORY EXPOSURE TO TEST AND REFERENCE SEDIMENTS

EXPOSURE SCENARIO*	REFERENCE	TEST
30/00	0	12
30/60	0	12
60/00	0	15
60/30	0	18

*Exposure senario is days exposure/days in clean water before pathologic assessment. The initial number of animals per test situation was 300.

TABLE 2

PERCENT FLOUNDER WITH HEMANGIOMA/OTHER PROLIFERATIVE LESIONS FOLLOWING LABORATORY EXPOSURE TO TEST AND REFERENCE SEDIMENTS

YEAR CLASS	100% REFERENCE	50% REFERENCE/50% TEST	100% TEST
0-1	00/00*	16/05*	25/13*
	33/03**	35/06**	25/22**
1-2	00/00*	17/25*	31/19*
	09/18**	09/09**	38/63**

 *Animals fed uncontaminated mussels.
**Animals fed mussels exposed to the test sediment.

Flounder, exposed four months in the laboratory to test sediment mixtures consisting of equal amounts of test and reference sediments, or food (mussels) previously exposed to test sediment, developed neoplastic or other proliferative lesions of the kidney, pancreas, external and oral epithelial surfaces. Of particular interest were pancreatic intra-insular neoplasms observed in fish exposed only to test sediment or to a combination of test sediment and being fed contaminated mussels. Animals exposed for 12 months developed hepatic megalocytosis, gastrointestinal adenomatous polyps and renal cystic adenocarcinoma.

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In Vitro Assays

Response data for reverse mutation in four strains of Salmonella, sister chromatid exchange in Chinese hamster V79 cells, and inhibition of gap-junctional intercellular communication in V79 cells are summarized in Table 3. Positive responses in Salmonella and for sister chromatid exchange were obtained only when extract exposure was combined with anexogenous microsomal (S-9 fraction of PCB-induced rat liver) metabolizing system.

TABLE 3

IN VITRO RESPONSE DATA OBTAINED WITH SALMONELLA AND CHINESE HAMSTER V79
CELLS FOR WHOLE AND FRACTIONATED (F1-F4) SOLVENT EXTRACTS
OF TEST AND REFERENCE SEDIMENTS

	REFERENCE SEDIMENT					TEST SEDIMENT				
	WHOLE	F1	F2	F3	F4	WHOLE	F1	F2	F3	F4
TA98	-	-	-	-	-	-	-	++++	+	+
TA100	-	-	++	+	-	++++	-	+++	++++	++
TA102	-	-	-	-	-	-	-	+	+	+
TA104	-	-	-	-	-	+	-	-	-	-
V79/SCE	+	-	-	-	-	++	-	++	-	-
V79/GJIC	-	-	-	-	-	-/+	-	-	-	++++

Response (R) is assessed in terms of increase over background (B).
(+) = $2.0B \leq R < 2.5B$; (++) = $2.5B \leq R < 3.0B$; (+++) = $3.0B \leq R < 3.5B$;
(++++) = $3.5B \leq R$.

DISCUSSION

The development of tumors in oysters exposed to test but not reference sediment, coupled with the occurrence of tumors in flounder for all exposure situations except those consisting of reference sediment plus uncontaminated food, demonstrate a causal relationship between tumor development in these organisms and exposure to the test sediment.

Whole and fractionated solvent extracts (F1-F4) of test and reference sediments were testable for mutagens in Salmonella and for sister chromatid exchange in Chinese hamster V79 cells. Most of the responses for genotoxicity were obtained with extracts of the test sediment and this correlated with the exposures required for tumor induction in experimental animals. The reference sediment generally contained the same chemicals as the test sediment, except at much lower concentrations. The fact that genotoxic activity was not detected with F1 is consistent with the types of compounds associated with this fraction (PCBs, aliphatic hydrocarbons and cycloalkanes, many chlorinated pesticides and two-ring aromatic hydrocarbons. Alternatively, the high level of genotoxicity associated with F2 correlated with the large number of

genotoxic and carcinogenic PAHs known to be in this fraction. Although poorly characterized chemically, F3 could contain substituted carbazoles and natural or substituted anthraquinones demonstrated to be genotoxic in some short-term tests (Tikkanen et al. 1983; Nishio et al. 1982; IARC 1983). F4 is chemically uncharacterized. The unqualified requirement for exogenous metabolism to detect genotoxic activity is consistent with the classes of genotoxic agents identified in the test sediment. This finding also supports the hypothesis that the sediments contained few (if any) direct-acting carcinogens. In general, greater genotoxicity was obtained with extract fractions than with whole extracts, suggesting antagonistic interactions in the latter.

The strong inhibiting effect of fraction 4 of the test sediment extract on gap-junctional intercellular communication in V79 cells suggest the presence of a potentially powerful, direct-acting, tumor promoter. This response was not due to known tumor promoters active in the assay (e.g. PCBs, chlorinated pesticides) as these would have been in other fractions. It has also been determined (Mills et al. 1988) that chemicals enhancing gap-junctional intercellular communication (e.g. 2,4-diaminotoluene) may reverse or suppress the inhibiting effects of other compounds (e.g. phorbol myristate acetate, aldrin, cyclohexylamine). The presence of such chemicals in complex mixtures may mask the presence of gap-junctional intercellular communication inhibitors. This phenomenon may explain the strong response with fraction 4 whereas little or no response was obtained with whole extract.

Overall, in vitro tests of sediment extracts may provide inexpensive and rapid approaches to problem diagnosis and identification of causal relationships. Although a good correlation was obtained between tumor induction in test species and in vitro test responses with the tumorigenic sediment, such tests may not be broadly predictive of carcinogenic effects (Tennant et al. 1987). Additional research is needed to establish the usefulness of in vitro tests with sediment extracts to predict carcinogenic effects in aquatic animals.

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IN VITRO MUTAGENICITY TESTING OF SEDIMENTS

by

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SUMMARY

o   A number of in vitro bioassays have been used successfully in the
    detection of mutagenic activity in polluted sediment.

o   The Ames test, which has been most frequently used, has had varying
    degrees of success in the detection of mutagenic activity in sediment.

o   Metabolic activation is a key component in the detection of
    mutagenicity in sediment. Incomplete metabolic activation likely
    accounts for variable results with the Ames test.

o   Although chemical extraction – concentration methods have been
    designed to recover PAHs from sediment, PAH levels in sediment do not
    correlate well with mutagenic activity.

o   As an alternative approach, the measurement of mutagenic activity in
    the bile of appropriate resident fish may be a means of detecting
    activity as well as fish exposure from mutagens originating in
    polluted environments.

CONCORDANCE OF SHORT-TERM GENETIC TOXICITY TESTS WITH THE
RODENT BIOASSAY RESULTS

by

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National Institute of Environmental Health Sciences

McCann et al. (1975) reported a high correlation (>90%) between carcinogenicity and mutagenicity among 300 chemicals tested in the Salmonella/microsome mutagenicity assay. Since that time, there has been a proliferation of short-term genetic toxicity tests that have been proposed to detect and discriminate between carcinogens and noncarcinogens. The National Toxicology Program (NTP) has evaluated over 250 chemicals for carcinogenic activity in the two-year, two-sex/species rodent toxicity assay. Approximately one-half of these chemicals were identified as carcinogens in at least one sex/species. This is an important data base because of the large number of chemicals that have been identified as noncarcinogens when administered at a maximum tolerated dose, in the same protocol as that which identified the rodent carcinogens.

Two recent studies have evaluated the capability of short-term tests to predict rodent carcinogenicity. The first study (Tennant et al. 1987) was undertaken to evaluate the predictive capability of four validated short-term genetic toxicity tests for 73 chemicals identified as either carcinogens (44) or noncarcinogens (29) in the NTP/NCI rodent toxicity bioassay. These chemicals were tested under code in the Ames Salmonella/microsome (SAL) mutagenesis assay, the assays for chromosome aberration (ABS) and sister chromatid exchange (SCE) induction in Chinese hamster ovary cells, and the mouse lymphoma L5178Y (MLYM) cell mutagenesis assay. The second report (Ashby and Tennant, 1988) assessed 222 chemicals (115 carcinogens, 24 equivocal carcinogens, and 83 noncarcinogens) for the correlation between chemical structural alerts (potential electrophilic 'DNA-reactive' sites), activity in the Salmonella/microsome assay and the level of carcinogenicity in the rodent toxicity assay.

The major conclusion of the first report (Tennant et al. 1987) was that no other single assay or any combination of the four assays exhibited a significantly greater concordance with the rodent bioassay than did the Salmonella/microsome assay by itself. The ability of the SAL assay to detect carcinogens (sensitivity) and noncarcinogens (specificity) was 45% and 86%, respectively. The positive predictivity of the SAL assay was 83% and the negative predictivity was 51%; the overall concordance with the rodent bioassay was 62%. Both the mouse lymphoma mutagenicity and sister chromatid exchange assays had significantly higher sensitivities (70% and 73%, respectively) than the SAL assay, but the specificities of both assays were 45% indicating that these assays incorrectly identified over half of the noncarcinogens in the data base as carcinogens. The SAL assay identified 20 of the 44 rodent carcinogens; all except one (selenium sulfide) exhibited a structural alert. Nineteen of these 20 chemicals were also identified in at least two of the other assay endpoints. Twelve of the 24 carcinogens that the SAL assay did not identify were also not identified by two or more of the other three endpoints. Five of the 24 carcinogens undetected by the SAL assay

exhibited structural alerts and three of these five chemicals were detected by all of the other three endpoints. The lack of 'structural alerts' and the inconsistent activity in the other genotoxic assays are characteristics that define the remaining 19 rodent carcinogens as being nongenotoxic. The overall concordance among the four short-term assays was high; that is, all four assays showed agreement for 33 of the 73 chemicals (45%); and three of the four tests were in agreement for an additional 26 (36%) chemicals. The negative predictivity for all four assays was nearly identical (50-52%), a value which is an indication of the significant number of nongenotoxic carcinogens in the data base. The range of concordance of the four tests with the rodent bioassay was 60-62%.

The results of the second study (Ashby and Tennant, 1988) demonstrated that there was a 90% correlation between the structural activity of a chemical, and activity in the Salmonella/microsome mutagenicity assay. Structural activity was defined by the presence of structural units or substituents (structural alerts) on a molecule that have electrophilic potential. The study confirmed that the SAL assay was a sensitive and cost-effective method of detecting the intrinsic genotoxicity of a chemical. Furthermore, a positive concordance between the structural alerts and the SAL assay activity of a chemical can be employed as an index of genotoxicity. Use of this index in the evaluation of 115 rodent carcinogens revealed two groups of carcinogens within the data base; those that were putatively nongenotoxic (41).

These two studies indicate that the Salmonella/microsome mutagenicity assay can identify the electrophilic/genotoxic potential of a chemical with a high degree of accuracy. A further strategy is required to confirm the carcinogenic potential of chemicals so identified. Several testing schemes have been proposed (Ashby 1986, Shelby 1988) that would utilize the in vivo chromosome aberration or micronucleus test in the bone marrow of mice or rats. Both of these endpoints are indicators of genetic toxicity.

Both studies have also clearly identified another group of carcinogens, the nongenotoxic carcinogens. Currently, this group of carcinogens represents between 35-40% of the rodent carcinogens identified in the NTP/NCI data base (Ashby and Tennant, 1988). At the present time there is no single test or battery of short-term tests that can discriminate between nongenotoxic carcinogens and noncarcinogens. This subject is of highest priority for continued research and development.

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APPENDIX II

SEDIMENT SUBCOMMITTEE MEMBERSHIP AND

TERMS OF REFERENCE

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APPENDIX II

SPANISH SCIENTIFIC LITERATURE AND

TERMS OF REFERENCE

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TERMS OF REFERENCE
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WATER QUALITY PROGRAMS COMMITTEE

BACKGROUND

Sediment can function as a sink for contaminants and also as a source of these contaminants to the water column and the biotic community. The magnitude and the conditions for the transfer of contaminants either way, is largely unknown. Therefore, it is difficult to establish the relative significance of the sediment as a sink or source of contaminants compared with other sources and pathways.

Previously, the focus has been on contaminated sediment in relation to dredging and disposal operations. This was the thrust of the Water Quality Board's Dredging Subcommittee, which largely completed its charge under its terms of reference. The current uncertainty over management and technical options available to address the issue of contaminated sediments affects the final resolution of most of the Areas of Concern identified by the Water Quality Board. In order to address the broader issue of sediment management in the Great Lakes ecosystem, the Water Quality Board has broadened the role of the Dredging Subcommittee to that of a Sediment Subcommittee.

TERMS OF REFERENCE

The Sediment Subcommittee will report to the Water Quality Programs Committee. The primary focus will be on management options for contaminated sediment in areas of concern and on the Critical Pollutants, as identified by the Water Quality Board. The Subcommittee will:

1. Review existing protocols designed to quantify the transfer of contaminants to and from sediments and to establish ecosystem impact. Based on this review, recommend protocols to be used or specific research to be undertaken to define the significance of these in-place pollutants in Areas of Concern.

2. Review existing technologies, including removal and disposal, treatment, capping and others for remediation of in-place pollutant impacts. Based on this review, evaluate the effectiveness, feasibility and costs of existing techniques. Report on the most promising and/or proven technologies for application to Areas of Concern and recommend technologies that should be further evaluated or demonstrated.

3. Maintain a registry where remediation of the contaminated sediments has been attempted or proposed. Use these examples for evaluating or demonstrating mitigative techniques.

4. Periodically review criteria and guidelines for the classification of sediments.

5. Maintain a register of significant dredging projects in the Great Lakes Basin with information on sediment volume and contaminant concentation.

6. Facilitate the exchange of sediment management information including, but not limited to, information relating to: development of sediment management technology; development of sediment evaluation protocols; procedures to characterize and quantify mass transport; and fate and effect of sediment and associated contaminants.

7. Identify research and information needed to remediate problems associated with contaminated sediment and encourage research and demonstration to investigate advances in sediment management technology and the pathways, fate and effects of sediments and sediment contaminants.

8. Develop a work plan for submission to the Water Quality Programs Committee, in accordance with the Board's planning and budget requirements. Review the work plan and revise as needed at least once a year.

9. Prepare periodic reports on the above items and undertake other activities as directed by the Water Quality Board.

MEMBERSHIP

Members of the Sediment Subcommittee shall be drawn from the jurisdictions or other organizations engaged in sediment management and related activities, and shall serve in their personal and professional capacity.

In consultation with the Water Quality Programs Committee, the Subcommittee may establish task forces to address specific sediment-related issues as need be. A chairperson shall be designated by the Water Quality Board for a two-year term.