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Project Summary

Health Hazard Evaluation of Waste Water Using Bioassays: Preliminary Concepts

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Effluents from waste water treatment facilities are discharged as a complex mixture of numerous chemical substances, which may include cytotoxic, carcinogenic and mutagenic compounds. Historically, Federal and State agencies have relied upon chemical-based analyses to set and enforce regulatory limits for these effluents. One problem with this approach is that many potentially hazardous chemicals may not be quantifiable in complex chemical effluents but are none the less discharged into the environment.

The U.S. Environmental Protection Agency has recently established a research program to determine if a bioassay approach for evaluating the potential adverse human health effects from exposure to complex mixtures might supplement conventional chemical analysis for setting regulatory limits for waste waters. The full report summarizes a bioassay testing strategy for characterizing cytotoxic and mutagenic activity of various waste water effluents. Use of a relative potency framework for assessing complex mixtures for potential health hazards is addressed.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The assessment of potential human health hazards from exposure to chemicals present in waste effluents is more difficult than the determination of toxic effects on the biota of receiving waters. The difficulties rest in several areas: the possibility of indirect, chronic exposure with low effective dose rates, the inability to test humans directly, and the difficulty in extrapolating between actual exposure and potential effects. The evaluation of potential human health effects involves an investigation of chemical specific toxicities or bioassays of whole effluents, a determination of the potential exposure to those agents, and a risk assessment based on the data generated.

Chemical specific analysis, which has traditionally been done in human health evaluations, requires the specific identification of pollutants and their concentrations in the effluent. Once identified these chemicals can be evaluated by consulting existing experimental and epidemiological data or by performing toxicological tests on surrogate organisms. This process has been applied to chemicals which are known to be present in an effluent which can be measured by standard chemical analyses, but the evaluation becomes more difficult for complex mixtures found in uncharacterized effluents. Synergistic or antagonistic interactions between chemicals can occur in the complex effluents. The process of screening for individual chemicals in complex waste effluents, even when limited to a list of priority chemicals can be tedious and expensive.

Toxicological assessment of the whole effluent may correct some of the disadvantages of chemical specific testing. Bioassays are utilized in this approach to estimate relative hazards from exposure to the chemicals in waste effluents. The use of the health-related bioassays as an approach to evaluating the hazards of chemical contamination in an uncharacterized waste effluent is discussed in the full report.

Biotesting Waste Water Effluents

Although it is recommended that all waste water samples be initially biotested directly without concentration, in practice a majority of samples will require some concentration before a positive response can be detected or a sample is declared negative. A major problem is that no currently available concentration procedure(s) can produce a concentrate from a water sample that contains all the original chemical constituents in an unaltered state and in the original relative concentrations. Moderately volatile and less volatile constituents may be tested as concentrates Concentration methods may be divided into the following two major groups:

Concentration: These methods refer to techniques in which water is removed and the dissolved substances are left behind. Examples of such methods are freeze concentration, lyophilization, vacuum distillation, and membrane processes such as reverse osmosis and ultrafiltration. A common disadvantage of these methods is that inorganic species such as sodium and chloride ions are concentrated along with the toxic constituents High concentrations of such salt species that are normally non—toxic can disrupt cell membranes through hyperosmotic effects.

Isolation: This group consists of processes in which the chemicals are removed from the water. Examples are solvent extraction and adsorption onto resins. Advantages of isolation methods include the fact that inorganic species are generally not concentrated. A disadvantage is that the isolation is selective in that neither solvent nor resin extraction will qualitatively and quantitatively yield all the organics present in a waste water sample.

After comparing the available methods against the optimal criteria for a sample processing procedure, the full report recommends that an EPA method using sorbent resins followed by organic elution and concentration would probably come closest to the ideal system for routine waste water processing for mutagenicity testing. A styrene divinylbenzene copolymer (XAD), was the recommended resin for concentration purposes. XAD resin can efficiently remove a large variety of model organic compounds from water.

"Blue cotton," a copper phthalocyanine derivative covalently attached to cotton, has been shown to be an effective adsorbent for mutagens with three or more fused aromatic rings in their structure. Although its limited adsorption properties prohibit the use of blue cotton in a comprehensive toxicological assessment of waste waters, it has features that could make it useful as a qualitative "spot" screen of potentially hazardous waste water sites. Blue cotton is simple to use and is amenable to batchwise extraction of water samples. Blue cotton can be contained in a mesh bag, suspended in the test water for the desired period of time, transported to the laboratory, rinsed with water, dried, extracted with ammoniacal methanol, concentrated and biotested.

Even when comprehensive testing is warranted, on-site concentration by collecting the sample through XAD columns has advantages. Problems associated with transporting and storing large volumes are avoided. The XAD columns and the small volumes required for sample characterization and for direct biotesting are relatively easy to handle.

In most cases toxic biological effects will not be detected in the unconcentrated water. However, the extent of concentration that should be done before a sample is judged to be safe is a difficult question to answer; these points are discussed in the report. In practice, toxicity sets upper limits on concentration. If the *in vitro* preparation is killed, it cannot be used for mutation of other assays. The volumes required for adequate biotesting plus the concentration factor set lower limits on the volume of sample that is required. For example, if a 1000-fold concentration factor is desired and a 10 ml volume of concentrate is required for the test procedures, then at least 10 L of sample must be concentrated.

Once the waste water effluents are properly collected and stored, chemically analyzed and concentrated if required, they are then subjected to bioassay procedures. Two procedures that have been successfully employed in directly biotesting waste water samples have been the *Salmonella* mutagenicity assay and the Chinese hamster ovary cell/HGPRT assay (CHO/HGPRT). Since environmental samples generally do not contain sufficient levels of contaminants to produce an effect in these two bioassays, the sensitivity of the two assays can be improved by increasing the size of sample volume or by concentrating the sample as described in the report.

In order to accommodate larger sample volumes and thereby biotest waste water samples directly, five times (5X) concentrated top agar and treatment medium have been used in the *Salmonella* and CHO/HGPRT assays, respectively. With this modification aqueous test sample volumes can be increased to 2.5 ml in the *Salmonella* assay and to 3.2 ml in the CHO/HGPRT assay, effecting a 17- to 25-fold concentration *in situ* relative to the standard (1X) assays. Control studies have shown the 5X and the standard assays to be equivalent when direct or indirect-acting standard mutagens are tested. *Salmonella* and CHO/HGPRT assays are outlined in the report.

When large volumes of environmental samples are assayed, certain properties of the samples become important. Microorganisms, when present, must be removed by filter sterilization. Particulates might also have adsorbed toxic chemicals. It is recommended that particulates be removed by centrifugation and/or filtration. In order to assess the biological activity of substances adsorbed to the particulates, the pellets and/or filters can be dried by lyophilization, extracted with dimethyl sulfoxide and assayed in the standard systems. The pH and ionic strength are also important properties to be considered when assaying large sample volumes. To avoid potential pH effects, the samples should be neutralized prior to being assayed. Hypertonicity considerations may limit the degree to which a specific sample may be concentrated.

Bioassay results obtained for a variety of unconcentrated waste water samples are described. The mutagenic responses observed to date have been greater in one *Salmonella* strain and do not require metabolic activation. Cytotoxicity to CHO cells was observed with waste effluents, but no mutagenic activity was observed in this cell line. One general observation of the data indicates that mutagenic chemicals may not be adequately removed by a municipal waste treatment facility.

Relative Toxicity Evaluation

Samples of complex mixtures can be evaluated by comparing toxicological responses to reference chemicals in a battery of biological test systems. Given equal biological response in a biological test system relative doses necessary to produce that degree of biological response may be used to evaluate the hazard represented by complex mixtures. Responses would reflect characteristics of both the sample being assayed and the test system used in the analysis

A relative potency framework could be used to evaluate complex mixtures as unknown test samples in comparison to reference chemicals. Relative potency (RP) is defined as

$$RP = \frac{\text{dose of reference material}}{\text{dose of test material}} = \frac{D_r}{D_t}$$

In a given biological system, responses to the test and reference materials must be compared in an equivalent test system. Reference materials are those chemicals that have been well characterized in terms of biological test

data. In such cases, it is suggested that biological results for test material can be indirectly related to health risk based guidelines or standards (S_r) of the reference material as follows:

$$RP_t \bullet S_t = RP_r \bullet S_r \text{ (but } RP_r = 1\text{), so}$$

$$S_t = \frac{S_r}{RP_t}$$

where S_t is the inferred guideline of the test material. The "leap of faith" implied in the use of such indirect methods for assessing potential risk results from the belief that short-term tests can be used as a scientific basis for decision making. Whether such a presumption is true has not been fully demonstrated at the present time and is a subject for further research.

Efforts to date have demonstrated that, in principle, a battery of short-term bioassays can be used to rank the relative hazard represented by chemicals which may be human carcinogens. The number of assays and the specific tests comprising a practical battery are not yet completely defined. Short-term test system results for a variety of materials should be incorporated into a data base that includes results for an inventory of chemicals and mixtures. Ultimately, it is envisioned that new results would be assessed by their position on a "relative toxicity scale." However, because of the small number of test results generally available from bioassays of waste water samples, variability due to experimental design and noise from random error, categorical assignments are currently preferred. For example, potential exposure via a water pathway will be excessive if the hazard index (HI) is

$$HI = \frac{R}{S_t} > 1$$

where S_t is a criterion or guidance value in units of concentration, and R is the measured or calculated concentration of the pollutant (compound or mixture) in the water sample at the point of consumption in the same units as S_t.

When the water sample is taken at a point of human consumption as opposed to a point of contaminant release, then dilution occurs between the release point and human exposure. Thus, R may be expressed as C_w/D where D is a generic dilution factor taken from EPA recommendations, and C_w is the concentration of a pollutant in the waste sample; thus, an unacceptable exposure could be present if

$$HI = R/S_t \leq (C_w \bullet RP_t)/(D \bullet S_r) > 1$$

D is the generic dilution factor, S_r is an EPA criterion for the reference chemical, and RP_t is the potency of the contaminant of concern relative to the reference chemical. The denominator (i.e. $D \bullet S_r$) models the change in concentration of the reference agent between points of release and consumption. The numerator (i.e. $C_w \bullet RP_t$) scales the measured concentration of the (tested) sample into an effective dose of the reference agent.

Since no definitive battery of assays has been identified as being predictive of human response, specific guidance for the composition of a battery cannot be offered at this time. However, a combination of bacterial mutation assays coupled with mammalian cell assays has been suggested by a variety of authors. A variety of different bioassays yielding positive results should be used. Constraints of time and money will probably limit the battery size to between three and six bioassays for most applications. Future work will hopefully identify the most useful battery of assays for the assessment of waste waters.

Conclusions

Regulation of waste water effluents on the basis of chemical analysis requires that all the chemicals which are present in a sample be identified and quantified and that the biological effects of those chemicals are known. The impracticality of meeting these requirements is evident. Short-term biotests can be useful and are probably necessary adjuncts to chemical analysis for waste water evaluation. Further work is necessary to identify the "best" short-term assay or battery of assays for biotesting waste water. Abundant evidence exists to indicate that short-term tests represent useful methods which need to be better validated for the biological assessment of the hazard posed by waste waters. It is recommended that research in this area be expanded, including the development of a data base devoted to results from the analysis of waste waters in a variety of short-term tests.

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The complete report, entitled "Health Hazard Evaluation of Waste Water Using Bioassays: Preliminary Concepts," (Order No. PB 88-243 860/AS; Cost: $19.95, subject to change) will be available only from:
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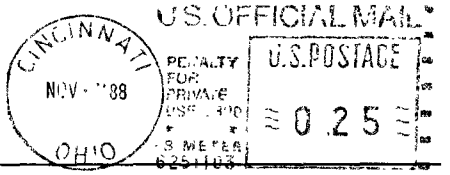

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