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A. F. Maciorowski

L. W. Little

L. F. Raynor

Ronald C. Sims

Utah State University

J. L. Sims

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Bioassays—
procedures and results

A. F. Maciorowski, L. W. Little, L. F. Raynor, R. C. Sims, J. L. Sims

Bioassay procedures to describe, evaluate, and predict the potential hazard of toxic materials to organisms and ecosystems, and the health-related aspects of polluted waters continue to receive widespread attention. Symposium proceedings and books pertinent to toxic substances management and test procedures have appeared, as have several literature reviews pertinent to specific pollutants. The proceedings of the fifth annual ASTM symposium on aquatic toxicology presented a collection of papers directed to research needs in aquatic toxicology, hazard assessment, new concepts in aquatic toxicology, biological availability and sediment toxicity, and hazard assessment and water quality criteria. A state-of-the-art overview pertinent to modeling the fate of chemicals in the aquatic environment propounded the concept that hazard assessment requires understanding the relationships between chemical concentrations that cause adverse affects on aquatic life and environmental exposure. This book featured definitions of chemical, physical, and biological processes that determine chemical fate; modeling of physical and chemical fate processes, water quality, microbial transformation and bioavailability; and modeling as a tool in assessing chemical hazard. Cairns et al. provided methods, advantages, difficulties, and future possibilities of biological monitoring in water pollution condensate into six categories including: early-warning systems; receiving system functional methods, relationships, and indexes; receiving system methodology based on community structure; toxicity testing; preference and avoidance studies; and future needs within the field as a whole. Fundamentals of freshwater pollution and its effects on living organisms, including an account of the work of the water industry in pollution control, were presented in a new textbook. Specific pollutants toxic to aquatic organisms were reviewed including petroleum and specific petroleum hydrocarbons, copper, zinc, lead, cadmium, chlorine residuals, halogenated hydrocarbons, oil well drilling fluids, and acidic deposition on aquatic ecosystems.

DESIGN, INTERPRETATION, STRATEGY

Various chemical, physical, and biological phenomena may alter chemical structure and therefore the fate and toxicity of certain compounds in natural and laboratory toxicity test systems. The biodegradation of five surfactants in relation to their toxicity to seven aquatic species was examined, and acute toxicity differed up to a factor of 1000 for the different organisms tested. However, different samples of one test compound may result in marked toxicity differences, and all compounds were biodegradable by more than 80% within 4 weeks. Bluzat et al. exposed Gammarus pulex to a high-level exposure of the fungicide Thiram for 1 hour, subsequently placed test organisms in freshwater, and compared mortality observed in organisms exposed to freshly prepared solutions to organisms exposed to the same suspension, 48 and 96 hours after preparation. Toxicity decreased 71 and 45%, respectively, in the 48- and 96-hour old suspensions. As demonstrated by such studies, physico-chemical data are often useful adjuncts for toxicity studies and environmental fate predictions. A laboratory method for testing the volatility of compounds from aqueous solution was described and the results discussed within the context of environmental behavior of chemicals. Key properties with regard to ultimate environmental fate included vapor pressure, water solubility, adsorption and desorption phenomena, partition coefficient, volatility, hydrolysis, and photochemical reactivity. Physicochemical factors influencing bioconcentration of organic solutes were examined suggesting a direct proportional relationship between bioconcentration factors and octanol-water partition coefficients. The correlation can be extended to give a simple relationship between bioconcentration factors (BCF) and aqueous solubilities. The relationships between physicochemical properties of 21 substituted phenols and their toxicity and accumulation in guppies were determined, and related to lipophilicity defined as log P from the I-octanol/water system, and to ΔPKa value. Results indicated that if LC50 values were corrected for ionization using an empirically formulated relation between toxicity and pH, the resulting regression could be used to predict toxicity for the pH range of 6 to 8. When corrected for ionization, log BCF of eight phenols was highly correlated with log P, but not with ΔPKa. Koch discussed structure-activity correlation of 10 chlorophenols as expressed by a molecular connectivity index (MCI) as a novel descriptor of molecular structure in a quantitative comparison with partition and biological properties of a molecule. The MCI was proposed as a way to predict the approximate acute toxicity values of environmental pollutants and toxic chemicals. Based on experiments with Mytilus edulis and organic chemicals, Geyer et al. suggested that water solubility and n-octanol water partition coefficients were useful screening tests for organic chemical bioaccumulation.

Data interpretation for bioassay research often requires a statistical approach. Calamari et al. applied a mathematical model of accumulation and elimination kinetics of Cr, Cd, and Ni in rainbow trout at levels approximating water quality criteria. The statistical estimation used parameter differences between single metal and mixture exposures and assumed that uptake and release are described by a reversible reaction as a two-compartment model. Based on PCB uptake experiments with plankton, a mathematical model was developed to predict PCB accumulation and determine the roles of feeding and sorption on uptake under various conditions. An allometric model for pesticide bioaccumulation was also reported using body size frequency distribution and appropriate allometric relationships to permit a more operational approach to pesticide bioaccumulation than the traditional trophic level concept. The qualitative similarity between experimental patterns and those in the literature from laboratory and field experiments suggested that empirical relations describing contaminant flux as functions of body weight form the basis for predicting contaminant body burdens in natural systems.
Jensen et al.\textsuperscript{24} employed a bioenergetic model to simulate PCB uptake by five fish species, which suggested that differences in PCB residues between and among species from different environments are related to exposure differences, size, rate of growth, and metabolic parameters.

Pollutants rarely occur singly in the environment, and quantification of joint toxicity remains of interest. The acute median lethal concentration of equitoxic mixtures of 8 and 24 toxicants was determined in a series of experiments and results expressed by means of a Mixture Toxicity Index.\textsuperscript{25} The toxicity of the mixtures was near concentration addition, and chemical concentrations near 0.1 of their LC50 value contributed to mixture toxicity. Schaeffer et al.\textsuperscript{26} used multiple regression analysis and the assumption of response additivity to obtain regression coefficients for mixture components and the mixture. A t-test was developed such that insignificant t values support additivity, negatively significant values support antagonism, and positively significant values support synergism. Heavy metal antagonism was also reported for two mollusk species. Sublethal concentrations of Se were found to decrease Cd-induced mortality in the snail \textit{Lymnaea stagnalis} by 50%\textsuperscript{,27} while the amount of Hg accumulated by the mussel \textit{Mytilus edulis} was decreased in the presence of Zn.\textsuperscript{28}

Correlations between laboratory toxicity test results and effects from actual environmental exposure require consideration of various biotic and abiotic factors. An Exposure Analysis Modeling System (EXAMS) designed for rapid evaluation of synthetic organic chemical behavior in aquatic ecosystems, combined loadings, transport, and transformation into differential equations using the law of mass conservation to allow computation of expected environmental concentration, fate, and persistence of chemicals.\textsuperscript{29} The Exposure Commitment Method for pollutant exposure evaluation was described as a time-independent approach to exposure evaluation measured as the concentration and duration of the presence of a pollutant in an environmental medium.\textsuperscript{30} The commitment method was said to be a convenient procedure for comparing contributions to intake and exposure from various pathways and in expressing source-receptor relationships. Frische et al.\textsuperscript{31} examined criteria to assess environmental behavior of chemicals with respect to selection and preliminary quantification. Factors of prime importance included the quantity entering the environment, mobility, accumulation, persistence, and direct or indirect toxic effects. These factors were quantified using production volumes, physicochemical data, results of degradation and accumulation experiments, and toxicity values. Quantified criteria for "well-known" organic chemicals were evaluated using appropriate weighing factors demonstrating that persistence and accumulation were most important; mutagenicity and carcinogenicity had to be strongly weighted; and mobility, although indispensable for estimating chemical distribution and persistence, was not included because it can have beneficial as well as adverse effects. A screening procedure to evaluate environmental behavior of chemicals by comparative evaluation of various parameters, termed the Ecotoxicological Profile Analysis, included consideration of bioaccumulation potential with algae and fish tests; mammalian retention and elimination with rat tests; information of biodegradability, metabolism, accumulation, and bound residues of the activated sludge test; and photomineralization by irradiation with UV light.\textsuperscript{32} The cumulative data are subsequently ranked and correlated with respective physicochemical properties, and test results compared to complete the assessment.

Dosing and monitoring methods in aquatic organism toxicity tests are often challenging, and Amelung\textsuperscript{33} described an apparatus for continuously saturating water with dissolved iron salts. The system used rainbow trout egg and larval development studies. An inexpensive device for continuous monitoring of dissolved gases and supersaturation termed a gasometer was described and may be used to activate an alarm system and, therefore, protect fish from hyperbaric or hypobaric gas pressures.\textsuperscript{34}

**MICROORGANISMS**

A compendium of microbiological aspects of water quality included various laboratory techniques, including bioassay procedures and screening tests.\textsuperscript{35} A bioassay method based on measurement of light output changes from luminescent bacteria was described as a practical and reliable method for toxicity monitoring.\textsuperscript{36} Busch\textsuperscript{37} described a system and test procedure to determine relative toxicity of water and effluent samples based on the biochemical oxygen demand (BOD) bottle bacterial test system. A procedure using oxygen uptake by bacteria in the presence of a pollutant was said to detect toxic responses within 40 minutes.\textsuperscript{38} Parker\textsuperscript{39} developed a microbial bioassay procedure that used active biomass and metabolic activities in terms of ATP and oxygen uptake that allows comparison of the microbial response to toxins with the response of a standard reference compound. This procedure was suggested as a surrogate parameter analysis for organic priority pollutants. The application of four bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures was examined, resulting in the conclusion that the screening tests had individual sensitivity patterns and that it seems unwise to use a single test for assessing the presence of toxicants in water or effluents.\textsuperscript{40} Trevors\textsuperscript{41} examined sensitivity differences between respiration, growth inhibition of active cells, and percentage mortality or resting cells using \textit{Pseudomonas fluorescens} exposed to pentachlorophenol, and growth was a more sensitive toxicity indicator than respiration.

Bacterial assays have been used to evaluate the potential impact of various substances to microbial species and communities. Studies on the effect of water soluble fractions from emulsified, degraded, and artificially weathered crude oil demonstrated that microbial uptake of glutamate was inhibited by emulsified oils, but not by oils that had been altered by biodegradation or weathering.\textsuperscript{42} Sayler et al.\textsuperscript{43} evaluated functional responses and recovery of sediment microbial communities using 20 estimates of microbial population density, biomass, and activity over a 15-month period. Multivariate analysis of variance and discriminant analysis were used to determine variation between contaminated sites. It was concluded that multiple functional measures of microbial community responses are required to evaluate the effect and recovery from environmental contamination, and certain physiological traits may not reflect population and biomass estimates of community response. The effects of PCBs on nitrification were examined using laboratory assays and it was determined that \(>10\mu\text{g/L}\) PCB inhibited nitrification in water from an unpolluted reservoir, but inhibition was not observed in pure
cultures or in water from a contaminated nutrient-rich reservoir.\textsuperscript{44} Mahaffey \textit{et al.}\textsuperscript{45} evaluated Kepone toxicity to mixed population of estuarine organisms and demonstrated that under anaerobic conditions, it reduced the number of colony forming units but had no effect on the number of organisms. The effects of carbaryl on four bacterial indicator species of fecal pollution and pathogenic bacteria were examined in pure culture and variously combined mixed cultures. Mixed cultures provided a better estimate of environmental effects of chemicals than pure cultures.\textsuperscript{46}

Trevors \textit{et al.}\textsuperscript{47} used a \textit{Pseudomonas fluorescens} assay to demonstrate that toxicity results were affected by the sequence of exposure to pentachlorophenol (PCP) and 2,3,4,5-tetrachlorophenol (TCP) including both the concentration used and the sequence of chlorophenol addition. Cell suspensions treated with PCP and subsequently removed from exposure were not affected by a second PCP dose; if the second dose was TCP the test species was sensitive to TCP. The toxicity of inorganic tin to estuarine sediment microbial populations was found to be influenced by a number of chemical factors.\textsuperscript{48} The use of gelatin and silica gel as a gelling agent decreased toxicity, cysteine had no apparent effect on toxicity, serine or 3-hydroxyflorone enhanced toxicity, while humic acids reduced toxicity. It was concluded that toxicity levels determined in the laboratory should be extrapolated to the environment with caution.

Bacterial growth is often used as a measure of productivity and eight methods of assessing growth rates were compared by Christian \textit{et al.}\textsuperscript{49} Linear and exponential growth rate constants were computed from cell density changes, bio-volumes, and ATP concentrations, cell division frequency, and RNA synthesis as measured by $^{3}P$ adenosine uptake. Estimates of \textit{in situ} bacterial productivity and growth were found to vary with the methods used and the assumptions regarding bacterial growth state. A bioassay method for determining the concentration of assimilable organic carbon (AOC) in drinking water distribution systems used growth of fluorescent pseudomonads as a function of AOC concentration.\textsuperscript{50} The method was used to examine water samples from various water treatment stages and piping material, demonstrating that AOC increased if water was ozonated or placed in contact with polyvinyl chloride pipes. Gordon \textit{et al.}\textsuperscript{51} assessed calorimetry as a technique for studying marine microbial metabolism and organic nutrient concentrations found in marine waters. Microcalorimetric measurements of heat production from glucose by \textit{Vibrio alginolyticus} demonstrated that glucose metabolism by this bacterium was measurable at submicromolar concentrations and that a constant metabolism efficiency was indicated over the wide range of glucose concentrations studied.

Determination of organic compound biodegradability has obvious implications for environmental toxicology. The reliability of the river die-away (RDA) test for establishing biodegradability of chemicals was assessed for reproducibility and RDA test results were not reproducible for di-2-ethylhexyl phthalate and phthalic acid in replicated tests with Missouri River water.\textsuperscript{52} It was suggested that RDA test biodegradation measurements are too variable and too dependent on laboratory treatment of samples to be applied directly to aquatic environments. Means and Anderson\textsuperscript{53} compared five different methods for measuring biodegradation (BOD, shake flask, CO$_{2}$ evolution, activated sludge, and the Gledhill test) using five organic compounds. Certain compounds were rapidly metabolized in all tests, while others degraded relatively rapidly in some and slowly or not at all in others. Inconsistencies were evaluated relative to the physical, chemical, and biological conditions inherent to each test.

Several investigators used microorganisms other than bacteria in bioassays. Babich and Stotsky\textsuperscript{54} examined the influence of abiotic environmental factors on nickel toxicity to mycelial growth rates of several filamentous fungi (\textit{Achlya, Saprolegnia, Cunnninghamaella, Aspergillus}). Nickel toxicity was eliminated with increasing pH and reduced in the presence of chlorophyll and humic acids; addition of Mg and Mn reduced Ni toxicity which was not affected by K, Na, Ca, or Fe additions. A reliable, accurate technique for identifying and analyzing solvent-pesticide interactions in bioassays was described that uses the fungi \textit{Pythium ultimum, Sclerotina homeocarpa, and Pestalotia sp.}, acetone, and the fungicides metalaxyl and captan.\textsuperscript{55} Interaction responses with any given set of bioassay parameters were dependent on both the acetone and fungicide concentrations, but the method compensates for discrepancies and indicates the most suitable test parameters to use in subsequent bioassays. A bioassay technique using the protozoan \textit{Tetrahymena pyriformis} was described that uses the rate of change of oxygen uptake, over a 10-minute period, as the endpoint.\textsuperscript{56} The procedure was not sufficiently sensitive to detect some chemicals at drinking water limits, but may be useful for industrial waste monitoring. The toxicity of the carbamate insecticide cartap was examined using a \textit{Paramecium primau-relia} assay providing a 24-hour LC50 of 2.5 mg/L.\textsuperscript{57}

**ASSAYS WITH ALGAE AND OTHER PLANTS**

New approaches to algal assays were proposed by several investigators. Domotor \textit{et al.}\textsuperscript{58} demonstrated that autoradiography facilitated detection of species-specific thermal stress in phytoplankton communities. An automated biological monitoring system based on changes in natural fluorescence of algae was designed and evaluated.\textsuperscript{59} Comparison of cell counts, \textit{in vivo} fluorescence, phaeophytin \textit{a}, and chlorophyll \textit{a} measurement as indicators of toxicity to \textit{Selenastrum capricornutum} showed that cell count data alone failed to indicate adequately toxic stress while \textit{in vivo} fluorescence gave an accurate and rapid indication.\textsuperscript{60} According to Sellner \textit{et al.}\textsuperscript{61} natural fluorescence can be enhanced by adding DCMU, suggesting that the almost instantaneous response of alga fluorescence to stress may provide a replacement for time-consuming algal assay procedures. Testing of volatile substances is difficult in the Algal Assay Procedure Bottle Test and a modification that maintains a constant concentration of volatile test substances in culture containers was devised.\textsuperscript{62} Algal assays continue to be used extensively in determining the limiting nutrient in waters and assessing eutrophication potential. Results of such studies were reported for Balaton Lake (Hungary),\textsuperscript{63} English lakes,\textsuperscript{64} Hastings Lake (Canada),\textsuperscript{65} the Rideau River (Canada),\textsuperscript{66} Lake Michigan,\textsuperscript{67} deep ocean waters,\textsuperscript{68} Memphremagog Lake (Canada),\textsuperscript{69} and Wolderwijd Lake (Netherlands).\textsuperscript{70} Young \textit{et al.}\textsuperscript{71} used \textit{Scenedesmus} bioassays to evaluate phosphorus availability in municipal wastewater. The problem of poor growth of many marine algae in
artificial seawaters was examined and it was suggested that selenium deficiency may be a problem.72 Visser and Couture73 demonstrated that Selenastrum capricornutum growth in water from natural sources was influenced by the nature and amount of the dissolved organics present. As a rapid response bioassay for nutrient additions under field conditions, Stephens and Shultz74 suggested measuring the ATP concentration of periphyton strips.

The difficulties in using a single bioassay method to determine nutrient status of a water were stressed by several investigators. Several bioassays for determining nitrogen and phosphorus demand were compared, and a combination of techniques allowed useful conclusions about nutrient status, but no single technique could be considered conclusive.75,76 Chiaudani and Vighi77 prefered a multistep approach to identify limiting nutrients. Lean et al.78,79 described the complicated interrelationships between light and nitrogen and phosphorus uptake and suggested that taken alone, results from 14C bioassays could be misleading. One reason for erratic results from 14C methods was the presence of trace metals in shock solutions and containers used in the assay, and procedures to eliminate such contamination were reported.80 Yallop81 obtained results that cast doubt on the validity of the light-dark bottle method of determining primary productivity. Because of the strong influence of "light history" on algal cell oxygen uptake, it was suggested that oxygen uptake measurements be made in the presence of dichlorophenyl-dimethylurea. Imaoka et al.82 used red tide organism algal assays to demonstrate that phosphorus removal from municipal wastewater was an effective control method.

Algae continue to be widely used in toxicity testing. Tests with various substances were reported including: snowmelt,83 leachate from Mount St. Helens ash,84 chlorine,85 pulp and paper wastewaters,86 coal liquids and oils,87-89 detergents,90 ammonia,91 hexa- and polychlorinated biphenyls,92,93 pentachlorophenol,94 and humic materials.95 Walsh et al.96 compared the sensitivity of Selenastrum capricornutum and Skeletonema costatum to that of Daphnia and Mysisopsis and to 10 wastewaters, and found algae to be more sensitive than crustaceans.

An apparent synergistic effect between the pesticides fenitrothion and aminocarb and the solvent used in their formulation was observed in bioassays with green algae and Daphnia.97 Elner et al.98 distinguished between effects on algal communities of aminocarb itself and those caused by other components of the commercial formulation Matacil. Selenastrum capricornutum assays were used to assess treatment efficiency in treatability studies of atrazine and dinoeb manufacturing wastewaters.99 Veber et al.100 suggested algae could be used to remove atrazine residues from polluted waters. Stratton and Corke101 found permethrin degradation products to be more toxic than permethrin to green and blue-green algae, and permethrin and its metabolites interacted differently in different combinations. In situ bioassays conducted in 60-L clear plastic bags were used by Hoffman et al.102 to evaluate effectiveness of algaicides in pond water. The order of effectiveness was simazine > diuron > copper > ethanolamine > copper citrate. The impact of herbicides on phytoplankton used in controlling aquatic macrophytes revealed that effects of 2-propanol varied for such species at different temperatures.103 Saroja and Bose104 found chlorophyll content to be the most sensitive indicator of methyl parathion toxicity to Chlorella. Effects of organochlorine insecticides on algae and other microorganisms were reviewed.105

Jouany et al.106 studied the toxicity of hexavalent chromium (Cr⁶⁺) to the green alga Chlorella vulgaris and the invertebrate Daphnia magna, both individually and in mixed culture using 24- to 96-hour and 28-day bioassays. Mixed culture results were substantially different than expected from single species experiments in that presence of Chlorella reduced Cr toxicity to Daphnia. Many investigations dealt with metal toxicity to several algal species. Five algal genera, as well as a natural phytoplankton assemblage were used in determining effects of a mixture of 10 heavy metals using primary productivity reduction and other responses.107 Foster108 used 200 isolates, representing 87 species of green algae, in sensitivity tests with Cu, Pb, Zn, and Cd, and found that isolates were generally resistant to metals normally present in their habitats. In related research,109 species of algae present in metal-polluted rivers seem to be determined by the degree of metal pollution. Fisher110 observed that conditions in a polluted estuary selected for heavy metal tolerance in marine diatoms. As part of a long-term study to evaluate the algal species composition trends in the Great Lakes, toxicity studies of heavy metals on various algal size fractions were conducted.111

The influence of Co, Ni, Cu, and Cd on Anacystis nidulans was examined to assist in response interpretation of blue-green algae at mining sites resulting in mutants tolerant to high metal levels.112 Of interest were synergistic and antagonistic effects noted with different combinations of metals. Interactions between Cd, Pb, and Ni in metal mixtures to three freshwater green algae were investigated,113 as were those of Se, Hg, and Cu in tests with Dunaliella.114 Petersen115 addressed the applicability of metal speciation in studies of the joint action of Cu and Zn on Scenedesmus quadricauda and proposed a model to account for observed biological effects as a function of metal speciation. Several studies concentrated on mechanisms of metal toxicity to algae, including surface adsorption of Zn,116 structure/toxicity relation of inorganic and organic tin compounds;117 the relationship of Hg chemical structure accumulation capacities118 and metal toxicity effects of pH with Chlorella.119

Generation time, maximum cell density, and chlorophyll content are commonly employed in assessing pollutant impact on algae. For the marine diatom Thalassiosira rotula, chain length was a useful indicator because the chains tended to break apart at toxic Cd and Ni levels.120 Usefulness of algae as biological metal monitors in the aquatic environment was investigated by Seelig and Cordazzo,121 who concluded that Enteromorpha was an effective Cu and Hg monitor, and by Burdon-Jones,122 who obtained unsatisfactory results with the brown algae Padina as a monitor for 10 metals.

The role of plants is becoming more common in toxicity, biomonitoring, and mutagenicity tests. Onion root tips were used to study biological effects of Cu in drinking water,123 and the aquatic plant Elodea was used in research on biological effects of Cu and methyl-mercury.124-126 Test results with Lemma demonstrated its similarity to animals in responses to pentachlorophenol.127 To facilitate use of macrophytes for monitoring stream water quality, Haslam128 developed a pollution index method. The potential of aquatic plants as indicators of metal pollution was demonstrated in Dutch,129
western Europe, and U. S. waters. For short-term genetic toxicity assays, the Arabidopsis plant seems to be suitable for in situ tests of soil- and water-borne pollutants. Italian researchers suggested that a root tip assay with the bean Vicia faba is sensitive, reliable, and inexpensive as a screening test for mutants. To assess effects of pollutants on seagrass, Walsh et al. devised a flow-through system containing whole plants or leaves of Thalassia testudinum. Plant genetic and cytogenetic assays with respect to protocols, use in screening programs, and recommended research have been discussed, while other studies have indicated that cultivated barley chromosomes are easily identified, making barley a suitable species for induction studies of chromosome aberrations. A screening technique based on differences between antibiotics and mutagens/carcinogens in the production mechanism of plastid-free Euglena mutants was used for rapid determination of mutagenic activity.

INVERTEBRATES AND FISH

Invertebrates and fish are widely used in bioassays to determine various lethal, physiological, pathological, and behavioral effects to the species under study. In this section emphasis is placed on procedural and methodological literature. For specific pollutant effects on marine and freshwater invertebrates and fish, the reader is referred to other titles contained in this annual literature review issue ("Bioaccumulation and Toxicity of Heavy Metals and Related Trace Elements," "Effects of Pollution on Freshwater Invertebrates," "Effects of Pollution on Freshwater Fish," and "Estuarine and Marine Pollution").

Freshwater cladocerans, specifically Daphnia magna, and D. pulex represent the most commonly employed freshwater invertebrate bioassay test species. A revised standard bioassay procedure for determining acutely toxic thresholds of soluble substances has been described, including culture techniques and test initiation and operation. The test is designed to determine the percentage of free swimming organisms at test termination, LC50 values are obtained by interpolation, and confidence limits are estimated using probability tables. An interlaboratory comparison of the D. magna survival/life cycle test was performed using silver and endosulphan as challenge toxicants, resulting in recommendations for procedure improvements.

Muller examined the interference of insoluble particles in the Organization for Economic Cooperation and Development (OECD) D. magna reproduction toxicity test protocol using various concentrations of silica gel, aluminum oxide, dextrane, and cellulose particles and concluded that the test is of limited value for insoluble test materials. Bowman et al. espoused conduct of Daphnia or Hyalella bioassays of dislodgable pesticide residues from foliage as an alternative to chemical analysis for the determination of safe worker reentry into crop fields treated with pesticides. Filtration rates and phototactic behavior of D. magna have been proposed as indexes of chronic Cu stress following comparison of these response with the more commonly used responses of survivorship, number of juvenile molts, age at reproductive maturity, and neonate body length. The former two responses and neonate body length were reduced at Cu concentrations that did not reduce longevity or reproduction, while juvenile molt number, age at reproductive maturity, and mean brood size responses were erratic to Cu exposure. To assess
icity test using ventilation frequency of steelhead as the response variable. Ventilation frequencies were enumerated from buccal and opercular activity. Ventilatory responses were detected at a Cu concentration of 144 μg/L as compared to a "safe" concentration of 444 to 819 μg/L determined in chronic exposure of embryos and juveniles, indicating that the ventilatory test was at least as sensitive as chronic toxicity tests. Thompson et al.\textsuperscript{156} demonstrated the applicability of time-series intervention analysis to determine the ventilatory reactions of bluegill sunfish exposed to sublethal Cu concentrations in fluctuating intermittent exposures.

Tests have also been developed to determine the presence and effects of carcinogens, mutagens, teratogens, and genotoxins on fish species. Laboratory and field studies of aquatic animals were reviewed indicating that fish have metabolic pathways similar to mammals for disposition of certain carcinogens.\textsuperscript{155} After screening 20 fish species, \textit{Boleophthalmus dussumieri}, an edible mud skippcr, was studied as a cytogenetic model for \textit{in vivo} mutagen detection.\textsuperscript{156} Utility of the killifish \textit{Nothobranchius rachowi} in a sister chromatid exchange test was described\textsuperscript{157} as was the use of sister chromatid exchange frequency in flatfish exposed to carcinogens.\textsuperscript{158} The suitability of a micorccus test for genotoxic compound depection using the eastern mudminnow \textit{Umbra pugmea} was also reported, and represents an alternative to chromosome aberration tests.\textsuperscript{159} Other physiological tests designed to indicate stress included: induction of mixed function studies (MFD) activity of the American eel \textit{Anguilla rostrata} as related to exposure time, clearance parameters, and dosing regimes for crude oil;\textsuperscript{160} induction of hepatic microsomal cytochrome \textit{P}$_{450}$ and aldrin epoxidase in the sculpin \textit{Leptocottus armatus} exposed to petroleum refinery wastewater;\textsuperscript{161} and the use of adenylate concentrations and adenylate energy charge as indicators of hypoxic stress in estuarine fish.\textsuperscript{162}

COMPARATIVE TESTING, MICRO COSMS, AND IN SITU TESTS

The realization that toxic chemicals may affect human health, as well as plants and animals in the environment has increased the concept of integrated assessment strategies to evaluate chemicals. Brusick and Young\textsuperscript{163,164} prepared two manuals related to Level 1 biological testing assessment and data format. The first consisted of a bioassay sensitivity literature review for validating test systems including the Ames test, rodent toxicity, \textit{in vitro} mammalian clonal toxicity assays, and consideration of the role of aquatic ecological and terrestrial ecological assays in environmental assessment. The second manual outlined the rationale and proposed methods for performing Level 1 health effects and ecological effects bioassays including methods for sampling and testing gases, suspended particulate matter, liquids, and solids. Specific assays for determining toxicity and mutagenicity using a variety of test systems and responses to assess potential environmental hazards were described. In addition to tests for mammalian, fish, terrestrial insect, aquatic crustacean, algal, terrestrial plant, and microbial toxicity, consideration of quality control for toxicity studies was discussed.

Short- and long-term toxicity studies with Cd were conducted with freshwater organisms from different trophic levels including bacteria, algae, crustacea, fishes, and amphibians, using a variety of lethal and sublethal endpoints.\textsuperscript{165} The lowest "no-toxic-effect-level" was determined to be 0.37 μg Cd\textsuperscript{2+}/L. Dauble \textit{et al.}\textsuperscript{166} examined effects of coal-liquor water soluble fraction (WSF) on growth and survival of two freshwater midges, a daphnid, and a green alga. Suppression of growth, reproduction, and survival was observed at concentrations of 0.02 to 0.42% WSF for the invertebrates, with algal populations being the least sensitive of the species tested. The acute toxic responses of species pairs tested simultaneously were determined for sodium lauryl sulfate, cadmium, and methanol.\textsuperscript{167} One species in each test was that recommended by the U. S. Environmental Protection Agency (EPA), the other a closely related species. Species pairs consisted of estuarine algal phytoplankters, mysid shrimp, copepods, and fish. For each toxicant, the species pairs yielded similar lethal or effective concentrations. Mysids were most sensitive to cadmium and methanol, with algae being most sensitive to sodium lauryl sulfate. Payne\textsuperscript{168} examined metabolism of oil spill surfactant compounds by rainbow trout, a crustacean \textit{Cancer irroratus}, and the mollusk \textit{Chlamys islandicus} demonstrating that each had the capacity for enzymatic hydrolysis of the complex fatty acid ester mixtures.

Comparative aquatic organism evaluation aids in assessments, but microcosm tests often allow more realistic simulation of environmental exposure conditions. Three-phase microcosm systems (sediment, water, and gas) for evaluating substances under a variety of ecological conditions were described by Porcella \textit{et al.}\textsuperscript{169} Response variables were related to general ecosystem functional processes (photosynthesis, respiration, anaerobic degradation, carbon cycling, and aquatic chemistry). A compartmentalized flow-through model ecosystem for studies of transport and degradation of pollutant has also been described.\textsuperscript{170,171} Microcosm-related mathematical models related to persistence were analyzed by Gard.\textsuperscript{172}

Several authors used microcosms to study effects of oil and oil-related constituents including effects of short-term exposure of crude oil and dispersants on benthic community metabolism,\textsuperscript{173} crude oil on simulated natural lakes using gas-water-sediment laboratory microcosms;\textsuperscript{174} the water-soluble fraction of a crude oil liquefaction product on laboratory pond microcosms;\textsuperscript{175} and no. 2 fuel oil on natural phytoplankton assemblages.\textsuperscript{176} Microcosm studies were also conducted to determine the significance of interfaces in the distribution and metabolism of di-2-ethylhexyl phthalate in a static laboratory ecosystem.\textsuperscript{177} Interface adsorption occurred rapidly and accumulations occurred in organisms inhabiting and/or feeding at the interfaces. Dickson \textit{et al.}\textsuperscript{178} used three-phase microcosms to study the aquatic fate and effect of benz(a)anthracene, which had no acute effect on aquatic organisms as measured by community structure and function. After 60 days, most benz(a)anthracene remained in the sediment with no evidence of metabolism. Atrazine toxicity to submerged vascular plants was investigated by Correll and Wu\textsuperscript{179} using simulated estuarine microcosms. Macrobenthic estuarine communities developed in sand-filled aquaria in the laboratory and field were exposed to chlorpyrifos to assess community structure effects.\textsuperscript{180} Molluscan larvae colonizing laboratory aquaria were sensitive at 0.1 μg/L, but later developmental stages characterizing field aquaria were not sensitive at 5.9 μg/L. Arthropod abundance was diminished significantly at the former concen-
tration in laboratory aquaria and at the latter concentration in field communities. Strachan et al. 181 developed a teflon chemostat for studies of trace metals to overcome problems of trace metal contamination from culture vessels and medium reservoirs. The only non-teflon components were glass pH and dissolved oxygen probes and short pieces of silicone rubber tubing that necessarily passed through the peristaltic pump.

A study of the biogeochemical fate and toxicity of Hg using controlled experimental ecosystems showed strong influence Hg affinities for organic matter in chemical speciation, transport, and toxicity. 182 The effects of Cu on an aquatic microcosm containing bacteria, algae, protozoa, rotifers, and oligochaetes demonstrated that stress responses were greater in early stages of heterotrophic succession and high nutrient concentrations. 183 The impact of Cd on freshwater bacterial communities in chemostats approximating natural river conditions resulted in measurable responses including bacterial productivity, accumulation by free bacteria, Cd budget in continuous culture, and Cd removal from the water phase. 184 Capone and Carpenter 185 developed a perfusion method for assaying nitrogenase activity in unmanipulated marine sediments and found more precise results than those obtained from conventional sealed-flask assays. Laboratory microcosms were used to study the effects of temperature and salinity. 186 and salinity and organic nutrient concentration, 187 on the survival and growth of Vibrio cholerae, which suggested that this species is an autochthonous member of the estuarine microbial community.

Microbial decomposition of the aquatic macrophyte Carex sp. was investigated in two microcosm studies, which suggested that pH reduction would significantly reduce rates of litter decomposition. 188, 189 Nutrient enrichment increased ATP levels associated with microbiota on fresh or partially degraded litter. The ATP increase occurred when microcosms were incubated in light, but not in the dark, suggesting that nutrient enrichment primarily stimulated the photosynthetic component of the detrital community.

Several reports dealt with the use of artificial streams for assessing toxicant effects in lotic ecosystems. Sanders 190 suggested design criteria for artificial stream facilities and discussed the strength and limitations of this approach. Specific applications of artificial stream research included an assessment of upwashes food quality from artificial streams dosed separately with chlorine, Cu, and dextrose. 191 Protein, carbohydrate, and organic content of upwashes samples were altered by taxonomic or physiological condition of the community. Hansen and Garton 192 evaluated the effects of diflubenzuron on a complex laboratory stream community demonstrating that insect fauna suffered direct toxic effects at 1.0 μg/L, but algal and fungal flora were only mildly affected at the same concentration. The effects of oil and gas drilling fluids on bimass and community structure of marine benthic microbial and invertebrate communities that colonize sands in running seawater were examined using biochemical methodology to assess impact. 193

In contrast to microcosm studies designed to simulate experimentally more natural conditions in the laboratory, in situ test methods provide an opportunity to examine toxic effects directly in the field. The dispersion, persistence, and biological effects of cypermethrin were evaluated following its application in a pond, which demonstrated that the insecticide was sorbed onto suspended solids. This permitted survival of fish, although high mortality of insects and crustacea was observed. 194 Application of 5 mg/L trichlorphenol and 1 mg/L pentachlorophenol into duplicate compartments of a natural experimental pond demonstrated that Daphnia populations were entirely eliminated after 8 days for the former and 3 days for the latter. 195 Both chemicals resulted in decreased autotrophic phytoplankton populations, increased flagellate and microorganism populations, and significant decreases in dissolved oxygen from balance shifts of autotrophic and heterotrophic populations. Tsushima et al. 196 reported that dioxin was reduced to 30% of its original concentration following pond application, but most of its metabolites were detected in the water column and aquatic vegetation. Treatment of an outdoor artificial pond with 15 μg/L permethrin indicated rapid loss of the compound from water and while readily absorbed by duckweed, residues were not persistent. 197 The long-term fates of three organochlorine pesticides were examined by dosing small experimental ponds. 198 Although chemically different, the residual behavior of the compounds followed a similar pattern resulting in high initial concentrations in biota, and a slow buildup and decline of sediment concentration. Three years after application of 50 μg/L of radiolabeled compounds, 14C residues of 0.1 mg/kg were detected in sediments and certain insects even though no detectable symptoms of poisoning were observed during the investigation period. Hildebrand et al. 199 determined the effect of the herbicide Roundup on rainbow trout in field experiments following operational application of 2.2 kg/ha, and 10× and 100× this field dose. Results indicated that no mortality was observed at any of the treatment levels. McLachlan and Hartz 200 surface dosed the supralittoral zone of an open sandy beach with crude oil, (weathered and fresh oil), fresh oil mixed with dispersions, and weathered oil at the water, and subsequently monitored meiofauna. Meiobenthos was reduced 1 month after dosing in all cases, but returned to normal in all cases by 5 months except for the site dosed with fresh oil and dispersant. McGregor 201 investigated factors affecting distribution of the bivalve Macoma balatica on a mudflat receiving wastewater effluent, and demonstrated that unchlorinated and chlorinated effluent was not toxic in laboratory tests, which was confirmed by performing 7-day in situ bioassays. The effect of experimental acidification (pH 4) on freshwater macroinvertebrate drift density in a mountain stream demonstrated that drift increased for the first 5 days, but no significant differences were observed between drift entering and leaving the acidified reach over longer periods. 202

HEALTH EFFECTS ASSAYS

Test development, evaluation, and validation of short-term bioassays for mutagenicity and cellular toxicity received intense investigation. A test approach using a battery of bioassays with careful selection of test systems similar to target organisms represents the current testing trend. Statistical analysis and interpretation of results of testing have also received attention. Evaluating, monitoring, and controlling the quality of complex toxicological research studies, particularly those dealing with toxic hazards of chemicals, were reviewed. 203 Kraybill 204 listed six specific factors that should be addressed in research studies designed to elucidate exposure/response relationships and risk analysis including: routes of exposure;
Water media; integrated exposure; fluctuations in contaminant levels; factors controlling absorption; and fluctuating exposure levels.

The mutagenic and carcinogenic evaluation of environmental pollutants, and several methodological problems including a critical evaluation of the Ames test, were discussed at the European Environmental Mutagen Society.187 Mutagenicity testing, bioassay procedures, and analytical techniques for detecting trace amounts of carcinogenic substances in environmental samples, were discussed at the 10th Annual Symposium on the Analytical Chemistry of Pollutants.206 Organization for Economic Cooperation and Development (OECD) guidelines for chemical testing were promulgated and procedures for laboratory testing of a property or effect deemed important for health and environmental hazard evaluation of chemicals were formulated.207 Projects under the auspices of the National Cancer Institute and the U. S. Environmental Protection Agency included effects of carcinogens, mutagens, and teratogens on aquatic species.208 Several tiered-testing scenarios were developed to screen chemicals that are potentially hazardous to public health.209 A systems approach for analysis of cost-effectiveness that uses sensitivity, specificity, accuracy, cost, and time for each screening test was presented. The development and direct application of short-term, rapid tests or assays in prokaryotic and lower eukaryotic microorganisms that screen for chemical carcinogenicity or mutagenicity agents were also presented.210 Systems of potential application for testing chemicals in water with emphasis on systems used or proposed for use by the Severn-Trent Water Authority Mutagenicity Unit (STMU) were discussed by Tye et al.211

Microbial mutagenicity tests, including the Ames, the polymerase A deficient E. coli, Saccharomyces cerevisiae D3, and Neurospora crassa tests were reviewed.212 Problems with response variation in the Ames assay may be caused by test strain preservation practices, choice of solvent, and dosages of test compound. Zeiger213 presented evidence that the apparent high correlation between mutagenicity and carcinogenicity in the Ames assay (up to 92%) is an oversimplification and may be misleading because different classes of chemicals show different levels of correlation.

Because no single test has been developed for detecting all hazardous chemicals, criteria for selecting the best combinations of tests for a test battery were proposed.214 This decision point approach involves five sequential steps in evaluating carcinogenic potential of a chemical. For estimation of carcinogenic potential of chemicals that have not been subjected to carcinogeniss assays, Enslein and Craig215 developed a statistical structure-activity equation based on substructural fragments and compound molecular weights. The accuracy of the carcinogen classification is between 87 and 91%, and between 78 and 80% for noncarcinogens. An approach for estimating the proportion of mutagens in a sample was based on discrete contagious distribution theory.216 Schaeffer et al.217 studied correlations between the number of mutagens, nonmutagens, and untested (mutagenicity) compounds identified in complex environmental samples. Concerning attempts to identify environmental mutagens based solely on DNA alteration, Zimmermann218 indicated that there are many primary targets where transmissible genetic damage can be induced, including enzymes of DNA metabolism, components of spindle-fiber apparatus, and membranes.

DeMarini et al.219 studied raw and treated waters from Lake Bloomington, Illinois, using four different mutation tests (Ames assay, mitotic gene conversion in strain D4 of Saccharomyces cerevisiae, Neurospora crassa forward mutation, Zea mays reverse mutation) and suggested that various genetic test endpoints should be used when testing drinking water. Pfeiffer220 reviewed the use of Salmonella and microsomes to detect mutagenic or carcinogenic substances in water. Water supplies of 12 Great Lakes municipalities were extracted using XAD-2 resin and tested for mutagenic potential with the Salmonella/mammalian microsome assay.221 Dose-related mutagenicity increases were found in 11 of the drinking water supply extracts. Using the Salmonella/microsome test for drinking water samples, Heartlein et al.222 concluded that drinking water contaminated with agricultural and/or industrial chemicals may result in a potential health hazard.

Because planned or unplanned potable water reuse may expose a population to some health risk, evaluating the potential toxicity of specific water sources is desirable. Neal223 suggested laboratory tests and monitoring procedures for evaluating effects of organic, inorganic, and radiological chemicals and particulate matter present in finished drinking water. Kalmaz and Kalmaz224 reviewed the health effects and ecological significance of chlorine residuals in water. Aldrich and Peoples225 found a correlation between malignant melanoma occurrence and trihalomethane contaminated drinking water supplies in Brevard County, Florida.

The Ames assay was used to assess mutagenic activity in fish (Rhinichthys cataractae) and sediment from the Sheep River, Alberta, Canada, which receives a discharge of chlorinated wastewater-works effluents.226 While there was a significant increase in the mutagenic activity in fish collected from the effluent plume, there was no evidence of increased mutagenicity in the sediments. Oncogenic effects of benzo(a)pyrene on three species of larval flatfish were investigated using concentrations comparable to levels found in polluted harbors.227 Adverse effects were found with sand sole and flathead sole eggs; however, English sole eggs did not show morphological anomalies. The DNA attacking ability using Bacillus subtilis, and mutagenic activity using the Salmonella typhimurium assay of aqueous sediment extracts of the Tama and Ayase Rivers, Japan, were investigated, and mutagenic and DNA attacking properties of samples confirmed.228 Suzuki et al.229 determined total mutagenicity of the lower Tama River sediment to be 10 times higher than the upper river sediment. Thin-layer chromatograms of the sediment fractions indicated that mutagenicity was attributable to polar, rather than polycyclic aromatic mutagenic compounds. The effects of advanced treatment on the mutagenicity of wastewater-works effluents were investigated with the Ames assay.230 Water chlorination and the occurrence of brominated compounds were correlated with mutagenicity. Ten wastewater work and industrial waste treatment plant effluents demonstrated mutagenicity and were found to contain 243 organic compounds, 20 of which are listed as priority pollutants.231 A mammalian cell culture technique was used to study the effect of water treatment processes on toxicity reduction of secondary effluents.232 Conventional water treatment was shown to be effective in removing toxicity.

As part of a continuing program to evaluate the toxicity of environmental samples with biological endpoints, Babish et
al.233 examined the lethality of municipal wastewater sludge organic extracts to mice. Extracts were observed to be extremely toxic, but toxicity did not correlate with PCB content or mutagenicity, and the relative hazard of toxic sludge components was difficult to assess. Hopke et al.234 demonstrated mutagenic responses in acetone extracts of anaerobically digested sludge using the Ames, Tradescantia paludosa, and Zea mays, and Bacterial assays.

Bacterial bioassays were used to screen for the presence of mutagens and toxins in extracts from groundwaters located in areas of in situ coal gasification.235 Mutagens were present in groundwater, persistent for 2 years after gasification had been terminated, and showed an activity change with time. Preliminary evidence suggested that quinolines and aniline derivatives were mutagenic in water, while the toxicants in groundwater were phenolic compounds. The environmental hazards from a laboratory-scale coal gasifier were reported, and the tar effluent stream was more toxic and mutagenic than aqueous condensate.236 Waste effluents produced by an experimental coal gasifier were assessed with the Ames assay, and aqueous effluents were mutagenic following metabolic activation.237 Ridlington et al.238 demonstrated hepatic microsomal cytochrome P450 oxidase induction in estuarine sculpin following in vivo exposure to Class B petroleum refinery effluent from two West Coast refineries. Using the Ames assay, frameshift mutagens identified in microbially degraded crude oil were not detected in crude oils prior to microbial attack.239 Mammalian liver microsomes were not used, as soil-degrading microorganisms constituted the enzyme activation system in the study. Brown and Donnelly240 quantified mutagenic potential of water concentrates from a waste oil storage pond effluent. Screening of 36 polynitroaromatic compounds in TNT wastewater was conducted using the Ames assay. Combinations of compounds to yield a synthetic condensate blend exhibited mutagenicity in five Salmonella strains (TA98, TA100, TA1535, TA1537, and TA1538) with and without metabolic activation.241 The reduction in mutagenicity of TNT-surfactant complexes has been compared with TNT and implications with respect to in situ treatment of contaminated lagoons and soils discussed.242

The mutagenicity of anthracene, anthraquinone, and four structurally similar compounds was evaluated by the Ames assay.243 The former did not exhibit mutagenic activity, but the other compounds were mutagenic for strain TA1537. In an investigation of the Ames assay mutagenicity of hydrazine derivatives and an aromatic amine, a qualitative correlation between the pKa of the compounds and their mutagenicity was demonstrated.244 Suzuki et al.245 reported the occurrence of mutagenicity by photochemical reactions of aromatic compounds such as polycyclic aromatics and monosubstituted benzenes in aqueous nitrate solution. Sayler et al.246 indicated that bacterial degradation of PCBs tends to decrease mutagenic potential of lesser chlorinated PCBs, and p-chlorophenylglyoxylic acid, p-chloromandelic acid, and three monochlorinated benzoic acids were not mutagenic in either the sister chromatid exchange or Ames assay.

Evaluation of the Ames Salmonella typhimurium/mammalian microsome mutagenicity assay with regard to significance of information obtained and modification of the assay received high priority in 1982. Friederich et al.246 attempted to determine the influence of parameters causing interlaboratory variation for Ames assay results. Parameters studied included histidine amount, sterilization of histidine and biotin, preparation of minimal medium, amount of minimal medium per plate, and plate storage time. Liver microsome S9 increased spontaneous background mutation frequency in the Ames assay in the absence of any added mutagen.248 Mitchell249 addressed the problem of establishing Ames test significance and discussed approaches and limitations in data manipulation and interpretation with respect to genetic significance. A set of mutagenicity data was analyzed using several different statistical procedures, and the appropriateness of the procedures compared to classical analysis of variance was discussed.250 Bernstein et al.251 described an empirical approach for analyzing Salmonella dose-response data, which assumed that the mutation rate at low doses is a linear function of dose. With the assumption of initial linearity, this method provided estimates of potency based on slope that were used to rank chemicals and compare potencies from different test systems to assess quantitative correlations. The ability of white-spotted char liver homogenate to convert chlorinated nitrobenzene ethers and their nitroso- and amino-derivatives to mutagens was investigated using the Ames assay, and results indicated that liver homogenate had the ability to convert these compounds to mutagens.252 The relationship between polynuclear hydrocarbon fluorescence on thin layer chromatography plates and mutagenicity assays indicated that samples demonstrating fluorescence were over five times as likely to be mutagenic than nonfluorescent samples.253

For several years, the activation of promutagens to mutagens has been a concern in the analysis and interpretation of mutagenicity tests. Gentile et al.254 suggested the use of radiolabeled promutagens in conjunction with assays of labeled metabolites with microbial genetic indicator organisms, as a technique capable of identifying specific genotoxic metabolites. A new screening technique in which complex sample components are separated by thin-layer chromatography and their mutagenic effect is registered directly on the plates by means of the Ames assay has appeared, and is useful for evaluating large numbers of environmental samples.255 A spiral plating method was used in conjunction with the Ames assay to reduce labor involved in the production of dose-response curves.256 With this method, nondiffusible and diffusible mutagens can be assayed. Karube et al.257 reported a microbial electrode system composed of an oxygen electrode and a membrane filter for retaining Salmonella typhimurium revertants. Preliminary mutagen screening was completed within a 10-hour period by correlating current decrease with mutagen concentration.

The usefulness and limitations of bacterial DNA-repair tests in mutagenicity/carcinogenicity screening were investigated in laboratory studies.258 Larsen et al.259 reviewed literature to determine the efficacy of DNA repair assays as screening techniques for mutagenic carcinogens. Despite the high frequency of positive reports, it was obvious that repair assays fail to detect, or detect with low efficiency, those agents whose main action is either intercalation or induction of strand breaks. Therefore, DNA repair as a basis for screening for mutagenic carcinogens was not considered useful. In another literature review, methodologies and status of the Host-Mediated Assay were evaluated and found to be an important mutagenicity/carcinogenicity test.260 With proper selection of protocols and indicators, valuable information can be obtained that would
be overlooked by strict in vitro assays. Efficacy of Aspergillus systems for determining chemical effects on mitotic segregation and mutation were critically summarized. Standardization of treatment procedures was recommended, as was the use of better-marked diploids.

Several assays were developed for environmental mutagens not efficiently detected by the Ames assay. Oberly et al. investigated the use of the LSU78Y Mouse Lymphoma Assay for detection of metal carcinogens. Evidence that the Bacillus subtilis mutigene mutagen screening test can detect mutagens that escape detection by the Ames assay has also been presented. Mutagenicity is indicated by an increase in the frequency of asporogenous colonies. The B. subtilis “rec assay” was studied in detail using an isogenic set of strains carrying different mutations in repair or recombination functions or both. Although the rec assay is not presently satisfactory for detecting promutagens, advantages of the assay include increased permeability of cells to chemicals, easy maintenance of culture strains, and the possibility of using purified spores rather than vegetative cells.

A. F. Maciorowski is with Ecological Analysts, Inc., Sparks, Md.; L. W. Little and L. F. Raynor are with L. W. Little Associates, Raleigh, N. C.; and R. C. Sims and J. L. Sims are with Utah State University, Logan. Correspondence should be addressed to Anthony F. Maciorowski, Ecological Analysts, Inc., 15 Loveton Circle, Sparks, MD 21152.

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Bioaccumulation and toxicity of heavy metals and related trace elements

Cornelius B. Murphy, Jr., Stuart J. Spiegel

The presentation of a review of bioaccumulation and toxicity of heavy metals requires the definition of the subject. In the previous review on this topic, 1 toxicology was defined by the Education Committee of the Society of Toxicology as "the science which studies the adverse effects of chemicals on living organisms and assesses the probability of their occurrence." With this definition in mind, the prime objective of any toxicology investigation is to provide the primary data base from which estimates of risk can be made for a given population of organisms. Heavy metals have been defined as those with a specific gravity greater than 4 or 5, located from atomic numbers 22 to 34 and 40 to 52 on the periodic table (as well as the lanthanide and actinide series), and having a specific biological response. The most common heavy metals include titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), silver (Ag), cadmium (Cd), tin (Sn), mercury (Hg), and lead (Pb).

Simkiss and Taylor 2 reviewed the assumptions and concepts involved in the use of organisms as bioaccumulators of metal ions. They conjectured that cells have evolved methods of solubilizing, transporting, regulating, detoxifying, and excreting metals in concentrations which they do require. If a metal acts as a pollutant it may act by disrupting normal homeostatic mechanisms. A series of test guidelines covering health effects, 3 environmental effects, 4 and chemical fate 5 was published. These documents were promulgated under Section 4(a) of the Toxic Substances Control Act (TOSCA), but may prove useful in the preparation of other toxicological protocols. A series of papers was published on aspects of biological monitoring. 6,7-10 For the purpose of extensive review of the field, biological monitoring was defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program," with the changes often from anthropogenic sources. 7

REVIEW SOURCES

The proceedings of the Fifth Conference on Aquatic Toxicology and Hazard Assessment reviewed recent progress in aquatic toxicology and hazard assessment. 11 Included were conceptual reviews in toxicology, including reports of the effects of methyl mercury on killifish, and cadmium and molybdenum on aquatic insects. Collected bibliographies with abstracts published by the National Technical Information Service were updated for the transition metals, 12 Pb, 13 As, 14 and Se. 15 These searches contain citations concerning toxicity, carcinogenicity, environmental pollution and other hazards and adverse effects of these metals. In addition, a compilation containing citations concerning heavy metal contamination of shellfish and marine plants was updated. 16

HEAVY METALS IN THE ENVIRONMENT

Studies conducted in Seattle evaluated the daily uptake of lead, cadmium, copper, and zinc from drinking water and the subsequent determination of components of variation 17,18. Metal concentrations were closely associated with the type of home plumbing. Sorensen et al. 19 evaluated morphological changes in adult green sunfish on exposure to aqueous solutions of arsenic as sodium arsenate using electron probe x-ray microanalysis. Cytoplasmic arsenic inclusions were observed that may be indicative of a direct association between arsenic and sulfur-rich protein molecules. Arsenic, along with 16 other metals, was investigated in the water, sediment, and biota characteristic of St. Louis Bay along the Mississippi coast. 20 Lytle and Lytle presented data for the clam, Rangia cuneata and oyster, Crassostrea virginica, in an effort to establish an