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Frank M. BUTTERWORTH, Lynda D. CORKUM and Judith GUZMÁN-RINCÓN (editors) (1995).
**BIOMONITORS AND BIOMARKERS AS INDICATORS OF ENVIRONMENTAL CHANGE: A HANDBOOK.**
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The purpose of this book is to document recent developments and applications in biomonitor and biomarker research. Monitoring the environment is absolutely essential if we are to identify hazards to human health, to assess environmental cleanup efforts, and to prevent further degradation of the ecosystem. Biomonitors and biomarkers offer a reasonable approach to making these assessments. Until now, monitoring has been based mainly on chemical observation and analysis, but the approach is problematic. Chemical monitoring being expensive cannot measure all pollutants and, given the complex array of contaminants in the environment, cannot provide good predictors of the effect of pollution on living organisms. Knowing lists of chemical contaminants is important, but not enough. However, interest in biomonitors and biomarkers has steadily grown as the value of such methods is realized in the surveillance of terrestrial and aquatic ecosystems.

The book resulted from a symposium of the same title that was held (June 5-9, 1994) as part of the annual conference of the International Association of Great Lakes Research held at the University of Windsor in Canada. Internationally renowned speakers, with expertise in the field of biomonitoring and biomarker technologies, were invited to present their recent work. The objectives were to: facilitate an exchange of ideas and knowledge on state-of-the-art biomonitoring methods and applications; develop associations between laboratories in signatory countries of the North American Free Trade Agreement (NAFTA) where there will be challenges of increased pollution in already threatened ecosystems; introduce researchers of the Great Lakes, the greatest natural water resource in North America, to the benefits of recent environmental monitoring technology.

El propósito de este libro es presentar las investigaciones mas recientes acerca del desarrollo y de las aplicaciones de los biomonitores y biomarcadores. El monitoreo del ambiente es esencial para identificar substancias dañinas a la salud, si se están evaluando los esfuerzos por limpiar el ambiente y así prevenir la degradación adicional del ecosistema. Los biomonitores y biomarcadores ofrecen una valoración cercana de estos impactos. Hasta ahora, el monitoreo se ha basado principalmente en la observación y en el análisis químico, pero la problemática va más allá de estos aspectos. El monitoreo químico no puede medir todos los agentes contaminantes que se encuentran en el ambiente, por lo que una buena predicción del efecto de la contaminación en los organismos vivos es limitada. Es importante, pero no suficiente, conocer la lista de sustancias contaminantes. Recientemente, ha aumentado el interés por el uso de biomonitores y biomarcadores como una alternativa para vigilar los ecosistemas terrestres y acuáticos.

El libro Biomonitores y Biomarcadores como Indicadores de Cambio Ambiental es el resultado de un simposio llevado a cabo en junio 5-9, 1994, que formó parte de la conferencia anual de la Asociación Internacional de Investigación de los Grandes Lagos, realizada en la Universidad de Windsor, en Canadá. Se invitó a reconocidos investigadores a nivel internacional expertos en el campo del biomonitoreo y del biomarcaje, para que presentaran sus trabajos más recientes. Los objetivos eran: facilitar el intercambio de las ideas y de los conocimientos sobre la aplicación de los métodos más avanzados en el biomonitoreo ambiental; desarrollar asociaciones entre los laboratorios de los países participantes del Tratado de Libre Comercio deAmérica del Norte (TLC) que presenta desafíos de una contaminación creciente en ecosistemas ya de por sí amenazados; presentar a los investigadores que trabajan en el ecosistema de los Grandes Lagos (el recurso de agua natural más grande de Norteamérica), las ventajas de la nueva tecnología en el control del ambiente.

## ABSTRACTS

Backer L., Grindem C. and Hunter J. PET DOGS AS SENTINELS FOR HUMAN EXPOSURE TO ENVIRONMENTAL POLLUTION. We have conducted an epidemiologic investigation of pet dogs to determine if pets can seve as sentinels for adverse human health effects associated with environmental contamination. The study area, a small town in North Carolina, contains a Superfund site in which there are five different areas contaminated with chlorinated pesticides as well as other agents. Control animals were selected from anearby town with no known hazardous waste sites. Pet dogs were recruited into the study by local veterinarians who identified animals that fit with our study criteria. Pet owners who consented to participate were requested to complete a questionnaire (to identify potential effect modifiers and confounders), and a 15 mL blood sample was taken from their dog. The following analyses were done: CBS, serum chyemistry profile, peripheral blood lymphocyte micronucleus assay (PBL MN) (using cultured PBLS), and lymphocyte subpopulation analyses (using flow cytometry). Preliminary evidence suggests that dogs living in the town with the Superfund sites exhibited higher PBL MN frequencies (27±11.9) and lower CD4-positive/ CD8-positive lymphocyte ratios (1.5±0.96) than the control population (11±3.67 and 2±1.16, respectively). Genotoxicity and immunosuppression can be biological manifestations of exposure to carcinogens and other environmental contaminants. Our studies suggest that populations living near hazardous waste sites may be at an increased risk of adverse biological effects, and perhaps adverse health effects, as a result of exposure to ambient contamination from these sites.

Bromenshenk J., Vicki J., Watson J. and Smith G.C. ASSESSING ECOLOGICAL RISKS IN TERRESTRIAL ENVIRONMENTS WITH HONEY BEES. Terrestial monitoring often relies on sampling several media – air, water, soil, vegetation, and food. A more efficient tactic is to use a mobile biomonitor that covers a large area, takes thousands of samples, and returns to fixed location where sampling and other measurements can be conducted. Honey bees (*Apis mellifera* L.) fit these criteria and are proven to be

useful as in *situ* monitors of contaminant exposure and associated effects. We have deployed bees as exposure monitors of contact, uptake, and fate of contaminant residues including trace elements, heavy metals, radionuclides, organic pesticides, and other organics. From these data, we produce isopol maps of chemical dispersion over landscapes and regions. With state-of-the-art computer equipment, we conduct accurate and precise field measurements of potential chemical impacts to bee populations. Because many factors can influence the results, cause and effect are difficult to establish. To address this issue, we developed PC BEEPOP, an ecotoxicological simulation model for honey bee populations. PC BEEPOP is intended to provide reasonably simple, but nonetheless realistic, predictions of population responses to environmental variability (e.g., whether conditions and forage availability) and contaminant exposure. By placing variables in the control of the user, the model and its associated toxicants database help clarify the outcome for environmental risk assessments. Comparison of simulated and actual colony population responses indicate that the model can predict outcomes, such as final population size, within the 95% confidence interval of actual mean colony populations.

Butterworth F.M. INTRODUCTION TO BIOMONITORS AND BIOMARKERS AS INDICATORS OF ENVIRONMENTAL CHANGE. Routine monitoring using biological test systems is critical in the surveillance of aqueous environments in order to identify hazards to human health. Biomonitors, in conjunction with chemical analysis, have traditionally been used to detect physiological, developmental, and genetic pathologies caused by toxic contaminants. However the environment is laden with complex mixtures of toxic pollutants which can produce complex biological effects requiring biomonitoring systems that can properly assess those hazards. Therefore there is an urgent need to accelerate research in developing improved and also new systems to detect existing and new hazards resulting from increased industrial development of the North American Free Trade Agreement (NAFTA) member countries. The major objectives of the symposium are to facilitate an exhange of ideas and knowledge on statr-of-the-art biomonitoring methods and applications. Further aims are to stimulate new associations and collaborations between laboratories in North America to respond to the challenges of increased pollution rates, and to propose biomonitoring standards and conditions equally applicable to the three NAFTA contries.

Chaudhry G.R. and Farrah S.R. ADVANCED AND RAPID METHODS FOR DETECTION AND IDENTIFICATION OF PATHOGENS IN LAKE WATER AND OTHER ENVIRONMENTAL SAMPLES. A number of microbial pathogens released by infected individuals or present in human and hospital wastes can potentially cause contamination of environmental systems including lakes used for recreational purposes. Such contamination of water resources may lead to human and environmental health problems. However, detection and identification of potential pathogens in environmental samples is often difficult. Similar to chemical pollutants the pathogens are quickly diluted in the environment, requiring large volumes of samples to be collected and concentrated for the detection of microbial infectious agents. The processing of such samples is laborious and time consuming, limiting the number of samples that can be analyzed. As a result routine monitoring of water resources is not feasible. Despite a rapid advancement in the detection of pathogens in clinical samples, efficient and sensitive methods for routine detection and identification of microbial infectious agents have not been available. However, with the advent of new biotechnology techniques, several advanced, rapid and efficient methods for detection of microorganisms in environmental samples have been developed and reported by several investigators. We have developed highly sensitive and rapid methods for investigation of viruses in environmental systems. Freshly obtained wastewater samples were concentrated using standard methods for enteroviruses. The viral concentrates and samples from other environmental systems were extracted for total nucleic acid in intact form using techniques developed during this study. The isolated nucleic acid was analyzed by *in vitro* amplification of the target sequences specific to infectious agents such as retroviruses and poliovirus. Another method involved the use of a biosensor electrode for the detection of viral agents. These and other advanced methods and their applications in routine monitoring of environmental systems including lakes will be discussed. Abstract only.

Corkum L.D., Ciborowski J.J.H. and Kovats Z.E. ADULT INSECTS AS BIOMONITORS OF ECOSYSTEM HEALTH IN THE GREAT LAKES AREAS OF CONCERN (AOC). We use adult aquatic insects *Hexagenia* burrowing mayflies (Ephemeroptera) and hydropsychidcaddisflies (Trichoptera) as naturally occurring biomonitors of sediment contaminants (heavy metals and polychlorinated biphenyls (PCBs)). Although routine monitoring of contaminants in aquatic stages of insects is difficult, adults are easily captured in large quantities using light or emergent traps. Because aquatic insects form an integral link in both aquatic and terrestrial food webs, body burdens of adults can serve as valuable indicator of ecosystem health. We describe a monitoring program for sampling AOCs with the most severe heavy metal problems, others with highest PCBs levels, and reference sites. Highest priority sites for inclusion will be those for which Stage I RAP reviews have been completed (i.e., ecosystem problems have been identified). Our role is primarily to coordinate procurement and distribution of equipment, training of personnel, timing of collections, and recovery/analysis of samples for groups in each AOC . Adult insects will be collected on shore using inexpensive UV light-traps operated by two people (local RAP volunteers) who have viewed a specially prepared training video tape. Samples will be packaged, stored frozen, and sent to a central laboratory for preparation and developed will be back-calculated from insect body burden concentrations. This monitoring program will provide an immediate platform to share data (computer-access bulletin board), build community support, and provide integrated, comparable data on the contaminant status of the Great Lakes AOCs.

Daly H., Sargent D. and Lunkenheimer L. OFFSPRING OF LABORATORY RATS FED LAKE ONTARIO SALMON ARE MORE REACTIVE TO NEGATIVE EVENTS. We previously reported that adult laboratory rats fed a 30% diet of Lake Ontario salmon for 20 days were more reactive to negative events than control rats fed Pacific Ocean salmon or no salmon. We have now shown that offspring of rats fed Lake Ontario salmon during gestation show the same behavioral changes when tested as adults, even though they were never fed the fish. They were tested in a contrast effect paradigm (shift from a large to a small food reward in a 185 cm runway) and progressive ratio task (press a lever 2, then 4, then 6 times, etc. to obtain a food reward), and resuls showed that they were more frustrated in both tasks than controls whose mothers had been fed Pacific Ocean salmon or no salmon.

Diaz-Barriga F., Yanez L., Ostrosky-Wegman P., Salazar A., Montero R., Gonsebatt M. and Gomez, H. ARSENIC EXPOSURE OF WORKERS AT A HAZARDOUS WASTE DISPOSAL SITE.

During operation, from November 1990 to July 1991, the site received 14,000 tons of hazardous waste. The analysis in some samples showed that the waste was rich in lead, chromium, mercury, nickel, and arsenic. For organic compounds, data was scarce, 4,758 tons were oils of solvents. The waste was deposited in drums and bulk material which were left outdoors. To evaluate human exposure twenty two workers were considered for the study, as they were the team that moved drums manually or discharged bulk material into the cells. When compared to a control group, workers showed a significant increment of chromosomal aberrations and higher levels of arsenic in urine and hair. We did not find statistical differences in urinary mercury, blood lead, phenol in urine, cadmium in hair or blood, sister chromatid exchange values, lymphocytes proliferation kinetics, and liver function tests. With data obtained from the company that administrates the site, it was calculated that lead was present in 67% of the wastes whereas arsenic in only 0.37% of them; however, the exposed population showed higher levels of arsenic but not of lead when compared to the control group. This could mean that arsenic is more bioavailable than lead. Another explanation could be that there are other sources of arsenic exposure for the workers. This is a remote possibility, as we can not invoke arsenic-contaminate seafood for this group, there are no industries in the area, and the levels of arsenic in water were below 10 µg/L.

Feyk L.A. and Giesy J.P. THE CAFFEINE BREATH TEST: A NOVEL APPROACH TO MONITORING CYTOCHROME P450-1A ACTIVITY IN BIRDS. Planar halogenated diaromatic (PHD) compounds such as some PCB and dibenzo-*p*-dioxin congeners induce the P450-1A cytochrome-requiring monooxigenase enzyme in birds and other animals. P450-1A activity is often used as a biomarker of exposure of wildlife to PHD, and is usually measured *in vivo* in liver tissue. We have developed a less invasive breath test to measure P450-1A activity in birds. This will allow an assessment of the activity of this enzyme without the need to kill birds, and will allow measurement on the same individual over time. Caffeine is specifically metabolized by the P450-1A enzyme. The breath test is performed by injecting $^{14}$C-labelled caffeine and measuring the $^{14}$C activity of respired $^{14}CO_2$ at time intervals. The breath test is an effective method for measuring P450-1A activity. Birds induced with TCDD metabolized the $^{14}$C-caffeine up to ten times more rapidly than controls. The results of field monitoring and recent laboratory trials with different caffeine substrates will be discussed. The potential for this type of assay will be discussed relative to other enzyme systems in birds.

Finch R.A., Shedd T.R. and Gardner H.S. THE DEVELOPMENT OF AN INTEGRATED BIOLOGICAL ASSESSMENT APPROACH TO ENVIRONMENTAL HAZARD ASSESSMENT. The U.S. Army is involved in the assessment and remediation of a number of hazardous waste sites. Chemical analyses alone are often insufficient for predicting the toxicity of the complex mixtures found at these sites. New toxicity assessment methods are under development for use in an integrated biological assessment approach to be used at these sites. At hazardous waste sites, the new methods will be used to monitor toxicity on-site, prioritize clean-up needs, improve clean-up criteria for contaminated soil and water, evaluate candidate waste treatment technologies, and provide post-remediation monitoring. The methods also can be used to test wastewater effluents to assess the adequacy of wastewater treatment methods and to detect and prevent spills of toxic materials. These new methods will be tested on-site in a mobile biomonitoring laboratory. Toxicity endpoints to be assessed on-site will include: Acute and Chronic Toxicity, Mutagenicity, Carcinogenicity, Immunotoxicity, Behavioral Toxicity, and Developmental Toxicity. The application of some of these new methods in a flow-through system in the on-site biomonitoring laboratory will be described. Abstract only.

Goering P.L. and Fisher B.R. STRESS PROTEINS AS BIOMARKERS OF EXPOSURE AND TOXICITY. Adverse environmental stimuli affect gene expression by enhancing the synthesis of stress proteins. This response has potential uses for *in vitro* toxicologic screening assays, environmental pollution monitors, and as markers of xenobiotic exposure and human disease. These uses must be validated with laboratory studies. We conducted a series of studies to explore whether chemical-specific or target-tissue specific changes in the *de novo* synthesis of stress proteins may represent biochemical "fingerprints" or "signatures" of exposure and toxicity. Cadmium and mercury were used as model hepatotoxicants and nephrotoxicants, respectively, and the effects of acute *in vivo* exposure on protein synthesis in target and non-target tissues in adult male rats were evaluated. *De novo* synthesis of stress proteins was analyzed by: 1) pulse-labeling proteins in liver and kidney slices with $^{35}$S-methionine, 2) SDS gel electrophoresis, and 3) autoradiography. Accumulation of stress proteins was assessed immunochemically by probing Western blots with monoclonal antibodies to specific stress proteins. Dose-and time-dependent changes in gene expression in liver and kidney were demonstrated after exposure to cadmium and mercury, respectively, as evidenced by enhanced *de novo* synthesis of the 70, 90, and 110 kDa stress proteins 2-4 hr exposure. The changes in protein synthesis were target-organ specific, i.e., the nephrotoxicant mercury produced changes only in kidney, and the hepatotoxicant cadmium produced changes only in liver. Effects on protein synthesis occurred prior to detection of liver and renal injury, using histopathologic, functional, and clinical indices. Thus, xenobiotic-induced changes in gene expression, including the enhanced synthesis of stress proteins, may represent biomarkers of exposure, toxicity, and organismal/environmental stress.

Gomez-Arroyo S. and Villalobos-Pietrini R. SISTER CHROMATID EXCHANGES AND CHROMOSOMAL ABERRATIONS IN *VICIA FABA* AS GENETIC MONITOR OF ENVIRONMENTAL POLLUTANTS. *Vicia faba* is an adequate test system to detect the effects of environmental pollutants, commonly used in cytogenetics because of its big chromosomes, high sensivity, and ease of manipulation. The root tips of *Vicia faba* were used to score the sister chromatid exchange (SCE) induced by the well water containing high levels of arsenic in several towns in the state of Coahuila in the Comarca Lagunera, Mexico. A 3h treatment was applied and the differential stain technique was used. Atomic absorption spectrophotometry showed that the arsenic concentrations in drinking water wells were between 0.11 and 0.695 ppm and, in all cases tested, the SCE frequencies were significantly different from the controls. In another study, chromosomal aberrations and centromeric alterations in the meristematic anaphase cells in the root tip of *Vicia faba* were registered in order to detect the cytogenetic effects of the superficial water and sediments of the Hydrological System of the Atoya-Zahuapan River in the state of Tlaxcala. The water was collected in nine sites and the primary roots were immediately introduced into it for a four hour exposure. After this they were rinsed and transferred to a bath with fresh water constantly bubbling for

aeration. Then they were fixed and stained with acetoorcein. Only in three of the nine sites the water collected in sediments produced significant levels ($p<0.001$) of chromosomal effects. Therefore, *Vicia faba* responds positively when it is used to detect the effects of an specific element as well as complex mixtures in water systems.

Gonsebatt M.E. LYMPHOCYTE PROLIFERATION AS A BIOMARKER OF ENVIRONMENTAL ARSENIC EXPOSURE. A human monitoring study was designed to further characterize the slower proliferation found in peripheral blood lymphocyte cultures from individuals, lifetime exposed to arsenic via drinking water. Blood and urine samples were taken from 25-30 volunteers living in a town where levels of arsenic in the drinking water were over 300 μg/L, and from a matched group of individuals with similar socioeconomic status but As levels in the drinking water are between 29-32 μg/L. Exposure was assessed by questionnaires and by determining the levels of arsenic in urine and water samples. Peripheral blood samples were used to start lymphocyte cultures stimulated with PHA; BrdU was added at 24 hours and cells were harvested at 36, 48,60 and 72 hours. Label, mitotic and replication indexes were scored blind on slides that were stained with anti-BrdU antibodies for label index and with the fluorescence plus Giemsa technique for the differentation of first, second, and third division cells, respecgtively. Exposed individuals showed lower label, mitotic, and replication indexes as compared with controls. Gender differences were also found. Correlations between label and mitotic indexes showed that progression from the initial S to M phase is altered in exposed individuals. Arsenic exposure as well as lead and mercury affect cellular immune response, which make the endpoint of cytotoxicity studied here adequate biomarkers to assess exposure.

Guzman J. *DROSOPHILA MELANOGASTER* SOMATIC MUTATION AND RECOMBINATION TEST AS A BIOMONITOR. The somatic mutation and recombination tests in *Drosophila melanogaster* were developed for the rapid and inexpensive detection of genotoxic agents in an *in vivo* assay in which somatic cells of a higher eukaryote are utilized. In the wing spot test, two different crosses involving the recessive wing cell markers mwh (multiple wing hairs) and $flr^3$ (flares) are currently used: (1) Standard cross; (2) Improved high bioactivation cross. The latter is characterized by an improved sensitivity to a number of promutagens and procarcinogens and especially to polycyclic aromatic hydrocarbons. The presence of different classes of spots can be due to different mechanisms: a) various types of mutational events (point mutation, deletion, specific types of translocations), b) mitotic recombination, and perhaps also c) monosomy. Besides, the analysis of two different genotypes (one with structurally normal chromosomes, another with a multiple inverted balancer chromosome) allows a quantitative determination of the recombinagenic activity of genotoxins. The SMARTs (for wing) are sensitive to a broad spectrum of genotoxic agents which belong to different chemical classes. They have been validated with approximately 290 compounds, and they have also been used for genotoxicity testing of mixtures which are part of the human diet, and for radiation. Recent results suggest that the larvae can be used as an *in vivo* nitrosation model. It has also been shown that the test is highly sensitive to the polycyclic aromatic hydrocarbons, widely distributed in the atmosphere. These characteristic demonstrate the suitability of *Drosophila melanogaster* as a biological monitor of environmental contamination.

Hasspieler B.M., Adeli D., Alipour M., Naji-Ali F. and Haffner G.D. GENOTOXIC POTENTIAL OF ENVIRONMENTAL POLLUTANTS IN THE GREAT LAKES BASIN: ASSESSMENT USING NEW HUMAN BIOASSAYS. There is an ongoing demand for the development of sensitive test methods to assess potential carcinogenicity of pollutants in humans. This has led to the use of a human hepatoma cell line, HepG2, for genotoxicity testing in our laboratory. HepG2 retains an adequate capability for xenobiotic biotransformation. Three assay methods were employed, namely the neutral red uptake assay for cytotoxicity, the alkaline elution assay for DNA single-strand breaks, and the unscheduled DNA synthesis (UDS) assay for DNA repair activity. Two test compounds were employed, namely methylmethane sulfonate (MMS) and 4-nitroquinoline *N*-oxide (4-NQO), a model nitroaromatic which is representative of numerous pollutant compounds. The aim of the study was to correlate genotoxicity of the test compounds, monitored as DNA single-strand breaks, with DNA repair activity monitored as USD. Significant increases in the UDS response were observed in the presence of 20 μM or 0.25 μM 4-NQO. In contrast, increases in DNA single-strand breaks, were only observed in the presence of 100 μM MMS or 1 μM 4-NQO. Furthermore, these higher concentrations were at or near levels which produced significant cytolethality. Therefore, induction of DNA repair expressed as UDS appeared to be a more sensitive indicator of genotoxicity in this test system. Further studies have recently been conducted on sediments collected from various sites in the Detroit river and Lake St Clair. Generally, the level of genotoxicity of sediment extracts correlated with the concentration of chemicals present. These studies will contribute further to the development of this human test system for the assessment of potential environmental genotoxins.

Hough R.R. AN INTEGRATED RIVER BASINS MODEL: A SYSTEMS APPROACH TO GLOBAL MONITORING. Technology enables the assembly of ever larger quantities of data and ever deepening data structures. To date, global models have been just global models. They have identified problems common to the peoples of the globe, without setting forth a basis or context for the specific actions carried out by specific peoples that could step toward a resolution of the identified problems. These developments are not sufficient. Human being must have stories, images, habits, and rules-of-thumb to live by and within complex environments. These informal guidelines are just not available as they relate to the globe which is shared by all. There is another way. Economics and ecology have always been deeply interconnected even if the interconnections have not been well understood or taken into account. Analysis of these related issues based on simple political boundaries does not lead to actionable insights on a global or local scale. There is growing agreement that river basin and islands are meaningful units for analysis. A holistic approach to those units allows the assembly of a global system. The division of the globe into river basins and islands results in some 734 regional entities which make both economic and ecological sense. Human populations in these units are cluster around key industrial facilities whose activities are central to the economic well-being of the populations. It is proposed that a global monitoring network be established to track events at those key facilities of which there are some 13,000. Currently available imagery and information can begin to enable such and effort and thereby provide a foundation for a new class of global model. Illustration of global views will be presented based on a 734 unit model under development.

Hudson L.A. and Ciborowski J.J.H. CHIRONOMID LARVAE (*DIPTERA: CHIRONOMIDAE*) AS MONITORS OF

SEDIMENT TOXICITY AND GENOTOXICITY. Chironomids are acknowledged as important indicators of the effects of sediment-bound contaminants. Changes in community composition can indicate impairment of natural ecosystems. In the laboratory, sediment bioassays with *Chironomus* are used to document cytotoxic effects on survival and growth. Evaluation of antennal or mourthpart deformities in both natural populations and bioassays suggest sediment teratogenicity. Other approaches are presently being developed to provide direct assays for genotoxicity. Chiromonids, like many other Diptera, possess giant polytene chromosomes in their salivary glands. Banding patterns can be used to characterize genetically unique but morphologically indistinguishable populations. Exposure of larvae to toxic compounds can result in stimulation or reduction in the size of puffs – site of RNA synthesis on these chromosomes indicative of rapid protein production. Toxicity-mediated puff responses occur independently of deformities. Other promising approaches include direct assays of DNA fragmentation and repair mechanisms, and detection of metallothionines indicative of metal stress. Successful application of these techniques in *Chironomus* provides a battery of tests that can simultaneously indicate cytotoxic stress, genotoxic stress, teratogenicity, and mutagenicity of sediments.

Ma T.H. *TRADESCANTIA* (SPIDERWORT) PLANT AS BIOMONITORS FOR THE GENOTOXICITY OF ENVIRONMENTAL POLLUTANTS. *Tradescantia*-Micronucleus (Trad-MCN) and *Tradescantia*-Stamen-Hair Mutation (Trad-SHM) assays are well established simple test systems for detection of genotoxicity of environmental agents for more than two decades. The procedure of the Trad-MCN assay includes the exposure of the plant cuttings which bear young inflorescences to mutagens, a 24 h recovery time, fixation of the flower buds and preparation of slide for scoring micronuclei in the early tetrads. The protocol of Trad-SHM assay is similar to that of Trad-MCN assay except that a longer recovery time of 7-11 days is required and the pink mutation events are scored in stamen hairs. *Tradescantia paludosa*, the clone 03, and two cultivated clones, 4430 and 02, have been extensively utilized as *in situ* biomonitors for the genotoxicity of water, air, and various gaseous mixtures; and as screening bioassays in the laboratory for chemical, physical agents and complex mixtures such as soil and sludge samples. Database of these bioassays compiled from different countries around the world including the Great Lakes and estuaries; and the latest findings from tests of contaminated soil, landfill gases, and leachates will be presented. These tests are also potential genotoxicity screening systems for sediment samples from the surface water and estuaries.

Metcalfe C.D., Balch G.C. and Arcand L. BIOMARKERS FOR EXPOSURE OF BROWN BULLHEADS TO CARCINOGENS IN HAMILTON HARBOUR SEDIMENTS. Brown bullheads (*Ictalurus nebulosus*) from Hamilton Harbour have high prevalences of epidermal and hepatic tumors. These neoplasms have been attributed to exposure to the carcinogenic organic compounds, including PAHs, that contaminate the sediments in some areas of the harbor. Bullheads with external neoplasms (papillomas) that were collected from the harbor had elevated hepatic AHH activity, and high levels of fluorescent bile metabolites and aromatic DNA adducts in hepatic tissue in comparison to reference fish. The pattern of these biomarkers closely resembled the responses observed in brown bullheads injected in the laboratory with organic extracts from Hamilton Harbour. An elevated incidence of hepatic micronuclei in Hamilton Harbour bullheads with external neoplasms indicated that these fish were exposed to genotoxic chemicals. These biomarker data strongly indicate that bullheads are exposed to the carcinogenic aromatic contaminants present in the sediments of Hamilton Harbour, and that this exposure is responsible for the development of neoplasms in these fish.

Pandey, P., McGowen, R.M., and Butterworth, F.M. WHOLE ANIMAL GENETIC BIOMONITOR OF ENVIRONMENTAL RECOMBINOGENS. The eye mosaic test of Vogel (1989, Mut. Res., 211:153), and Vogel and Nivard (1993, Mutagen, 8:57), in *Drosophila* is a comprehensive, whole-animal genotoxicity test. Also known as the SMART (somatic mutation and recombination test) assay, it mainly detects changes in the rate of mitotic crossing over occurring during the larval stage in rapidly proliferating cells of the adult eye. The test also detects unequal sister chromatid recombination, gene conversion, X-chromosome loss, and deletion of or mutation in the *white (w)* reporter gene. The test also shows good validation with the rodent carcinogen assays. An important feature of this unique assay is that levels of xenobiotic metabolism can be genetically regulated. Some strains are suppressed and others are constitutively over-expressed. Exposure to test material can be acute, by placing the larvae directly in the contaminated medium, or chronic, by feeding the pollutants directly. Experiments in our lab indicated that bioactivation products of 4,4'-dichlorobiphenyl were recombinogenic. These metabolites have been extracted from the high bioactivation animals and are being identified. Experiments to monitor for recombinogenic effects of complex mixtures of contaminants in soils and river sediments are underway. The significance of recombination having far more impact than single gene mutation will be discussed.

Petras M.L. and Vrzoc M. BIOLOGICAL MONITORING OF ENVIRONMENTAL TOXICITY IN SOUTHWESTERN ONTARIO. Continuing contamination of our environment has resulted in a need for a system to monitor changes in environmental genotoxicity *in situ*. To this end both wild and laboratory mice (*Mus domestic*) have been used as sentinel organisms. Two procedures, the Sister Chromatid Exchange (SCE) test and the MicroNucleus (MN) assay for peripheral blood erythrocytes have been adapted for effective *in situ* monitoring of genotoxicity. In applying these procedures to samples of natural populations factor such as sex, age, stress, and genetic heterogeneity which might affect their reliability have been examined. Monitoring of natural populations of house mice inhabiting corn cribs in southwestern Ontario showed both seasonal and geographic patterns in five years of SCE data. No obvious patterns were, however, observed in two years of MN data. The seasonal patterns were related to the spraying of crops with herbicides. Three time periods can be defined: prespraying (April and May), spraying (June and July), and postspraying (August and September). The specific dates of the periods vary from year to year depending on the conditions for planting. In most years the lowest SCE values were seen in the prespraying period and the highest in mice collected during the spraying season. Geographically there are two main regions: Essex County (cribs within 50 km of the Detroit-Windsor complex) and Kent County (cribs 90 or more km from the Detroit-Windsor area). Generally mice from the latter have fewer SCEs than do mice from the former. There were some exceptions. Besides revealing the existence of frequency patterns these studies also provide "baseline values" against which future findings may be compared.

Sgro G. and Johansen J. ECOLOGY AND ASSESSMENT OF THE ALGAE OF FOUR LAKE ERIE ESTUARIES. Composition of algal periphyton is presented for Old Woman Creek, Black River, Cuyahoga River, and Ashtabula River estuaries. Samples for this study were collected from grab samples and on glass slides from diatometers for November, 1992, and April, 1993. Algal community structure and water quality assessments were made on the basis of standard and consistant identification and enumeration techniques, species diversity, relative abundance and global autoecologies. Statistical and multivariate techniques were used to compare the samples. For combined samples 258 algal taxa were identified. Generally, these estuarine systems were supporting stressed algal periphyton communities. Typically, these communities had low diversity and were dominated by cosmopolitan, pollution tolerant species. The greatest departure from this pattern was found in the November Ashtabula River samples and the November Old Woman Creek samples.

Tice R.R. ELECTROPHORESIS ASSAY: A VALUABLE TOOL FOR THE DETECTION OF DNA DAMAGE IN AQUATIC AND TERRESTRIAL ORGANISMS. An alkaline microgel electrophoretic technique has been developed which permits the detection of DNA damage (single strand breaks, alkali-labile sites) in individual cells (Singh *et al.*, Exp. Cell Res. 175, 184, 1988). Eukaryote cells are embedded in an agarose gel on a microscope slide, lysed by detergents and high salt, exposed to alkali (pH>13), and then electrophoresed under alkaline conditions. Cells with increased DNA damage display increased migration of the DNA from the nucleus toward the anode. The importance of the SCG technique lies in its ability to detect intercellular differences in DNA damage in virtually any eukaryote cell population, its sensitivity (detection limit of ~0.05 Gy gamma rays), its requirement for extremely small numbers of cells (<10,000 per sample), and its technical simplicity. The factors make the technique uniquely suitable for environmental biomonitoring studies. In terrestial systems, we have demonstrated increased levels of DNA damage in cells sampled from bone marrow, brain, and liver of mice (*Ochrotomys nuttalli* ) live-trapped at an EPA Superfund site. For aquatic system, we have developed methodologies for evaluating DNA damage in various organs (gill, liver, kidney, and spleen) of medaka (*Oryzias latipes*), and in tissue sampled from clams and mussels.

Tracy D., Mary, Freeman C.R., Anton Hough and Emlen J.M. MEASURING STRESS ON AQUATIC MACROPHYTES USING DEVELOPMENTAL STABILITY. Developmental stability has been used as an indicator of stress for a variety of organisms subjected to an array of stressors. While most traditional parameters quantifying stress on an organism are based on growth, developmental stability is based on the regulation of growth. It investigates the variance of a trait, which can increase greatly before the mean of a trait is affected. Investigating developmental stability is cost-efficient, time-effective, non-destructive and extremely sensitive. We conducted a pilot study on the alga, *Fucus furcatus latifrons*, growing at sites that varied in levels of organic pollutants. Its developmental stability, measured by fluctuating asymmetry of frond branch length, fractal dimension, and frequency of phenodeviants was contrasted with the traditional parameter, branch length. While a significant difference was detected among polluted and non-polluted sites for fluctuating asymmetry ($F=0.56$, $P<0.76$) did not differ significantly. This supports the use of developmental stability as an indicator of stress.