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Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing

A Bibliography with Abstracts

TO ASSIST IN:

- REFINING EXISTING TEST METHODS
- REDUCING ANIMAL USAGE
- REPLACING ANIMALS AS TEST SYSTEMS

PREPARED BY

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The Scientific Community, concerned about animal welfare, is sensitive to concerns regarding how and why animals are used in biomedical research and testing to evaluate the toxicological potential of various substances. Although alternatives to methods based on the use of animals may not satisfy all requirements and needs of the biomedical research and toxicologic testing communities, alternatives to the use of vertebrates are being developed and evaluated. Research on such methodologies is aimed at refining procedures to reduce pain and discomfort; reduce the number of animals required to provide scientifically valuable results; and to replace live vertebrates when an alternative methodology can be verified and validated by the scientific community.

The purpose of these bibliographies on "animal alternatives" is to provide a survey of the literature in a format which facilitates easy scanning. This bibliography includes citations from published articles, books, book chapters, and technical reports. Citations to items in non-English languages are indicated with [ ] around the title. The language is also indicated. Citations with abstracts or annotations relating to the method are organized under subject categories. This publication features citations which deal with methods, tests, assays or procedures which may

prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled through searching various computerized on-line bibliographic databases of the National Library of Medicine, National Institutes of Health.

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Suggestions and comments are welcome.

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REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

MISCELLANEOUS

GENERAL

Advancing refinement of laboratory animal use. Lab Anim 1998;32(2):137-42.
Whatever view is taken of the morality of using animals in scientific research and safety testing, it can generally be agreed that so long as such use continues, every effort should be made to keep animal suffering to a minimum. This is the thinking behind the 'Three Rs' of refinement, reduction and replacement of laboratory animal use. This paper concerns refinement. We recognize that the Three Rs are taken very seriously in many countries of the world [see for example a recent editorial in the journal Science (Goldberg et al. 1996)] and, although we have written this paper from our own perspective in the UK, its principles are generally applicable.

Balls M.**Mechanistic approaches and the development of alternative toxicity test methods.**Environ Health Perspect 1998;106(Suppl 2):453-7.
A mechanism can be defined as an explanation of an observed phenomenon that explains the processes underlying the phenomenon in terms of events at lower levels of organization. A prerequisite for new, more mechanistic, approaches, which would use in vitro systems rather than conventional animal analogy models, is a strengthening of the underlying scientific basis of toxicity testing. This will require greater recognition of the differences between fidelity and discrimination models and between analogy and correlation models. The development of high-fidelity, high-discrimination tests with a sound mechanistic basis will also require greater appreciation of the interdependence of all the components of test systems and the development of new alternative (i.e., nonanimal) testing strategies that can provide the specific knowledge needed for making relevant and reliable predictions about the potential effects of chemicals and products in human beings. The optimal use of this new knowledge will require fundamental changes to current practices in risk assessment.

Blaauboer BJ, Balls M, Barratt M, Casati S, Coecke S, Mohamed MK, Moore J, Rall D, Smith KR, Tennant R, et al. **13th meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): alternative testing methodologies and conceptual issues.** Environ Health Perspect 1998;106(Suppl 2):413-8.
Substantial world-wide resources are being committed to develop improved toxicological testing methods that will contribute to better protection of human health and the environment. The development of new methods is intrinsically driven by new knowledge emanating from fundamental research in toxicology, carcinogenesis, molecular biology, biochemistry, computer sciences, and a host of other disciplines. Critical evaluations and strong scientific consensus are essential to facilitate adoption of alternative methods for use in the safety assessment of drugs, chemicals, and other environmental factors. Recommendations to hasten the development of new alternative methods included increasing emphasis on the development of mechanism-based methods, increasing fundamental toxicological research, increasing training on the use of alternative methods, integrating accepted alternative methods into toxicity assessment, internationally harmonizating chemical toxicity classification schemes, and increasing international cooperation to develop, validate, and gain acceptance of alternative methods.

Bruner LH, Carr GJ, Curren RD, Chamberlain M. **Validation of alternative methods for toxicity testing.** Environ Health Perspect 1998;106(Suppl 2):477-84.
Before nonanimal toxicity tests may be officially accepted by regulatory agencies, it is generally agreed that the validity of the new methods must be demonstrated in an independent, scientifically sound validation program. Validation has been defined as the demonstration of the reliability and relevance of a test method for a particular purpose. This paper provides a brief review of the development of the theoretical aspects of the validation process

and updates current thinking about objectively testing the performance of an alternative method in a validation study. Validation of alternative methods for eye irritation testing is a specific example illustrating important concepts. Although discussion focuses on the validation of alternative methods intended to replace current in vivo toxicity tests, the procedures can be used to assess the performance of alternative methods intended for other uses.

Curren R, Bruner L, Goldberg A, Walum E. **13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): validation and acute toxicity testing.** Environ Health Perspect 1998;106(Suppl 2):419-25.
Scientific principles demand that before newly developed alternative methods for safety testing are fully embraced by the industrial or regulatory community, they reliably and reproducibly predict the designated toxic end point. The process used to determine reliability and reproducibility is termed validation, and it generally culminates with a highly controlled, blinded study using multiple chemicals and laboratories. It is imperative that the validation study is designed to confirm the previously established reproducibility and predictive power of the assay. Much has been learned recently about the practical aspects of validation through investigation of alternative methods for acute toxicity testing, i.e., those methods that assess acute systemic toxicity, skin irritation, and eye irritation. Although considerable progress has been made--many alternative tests are now commonly used in various industrial settings--there have been few tests that have successfully passed a complete validation. Some of the barriers to successful validation have been a) lack of high-quality, reproducible animal data; b) insufficient knowledge of the fundamental biologic processes involved in acute toxicity; and c) the development of truly robust in vitro assays that can accurately respond to materials with a wide range of chemical and physical characteristics. It is recommended that to progress in the areas of eye and skin irritation we need to expand our knowledge of toxic markers in humans and the biochemical basis of irritation; progress in the area of acute systemic toxicity will require the development of in vitro models to determine gastrointestinal uptake, blood-brain barrier passage, and biotransformation.

Davila JC, Rodriguez RJ, Melchert RB, Acosta D Jr. **Predictive value of in vitro model systems in toxicology.** Annu Rev Pharmacol Toxicol 1998;38:63-96.
The application of in vitro model systems to evaluate the toxicity of xenobiotics has significantly enhanced our understanding of drug- and chemical-induced target toxicity. From a scientific perspective, there are several reasons for the popularity of in vitro model systems. From the public perspective, in vitro model systems enjoy increasing popularity because their application may allow a reduction in the number of live animals employed in toxicity testing. In this review, we present an overview of the use of in vitro model systems to investigate target organ toxicity of drugs and chemicals, and provide selective examples of these model systems to better understand cutaneous and ocular toxicity and the role of drug metabolism in the hepatotoxicity of selected agents. We conclude by examining the value and use of in vitro model systems in industrial development of new pharmaceutical agents.

Lagadic L, Caquet T. **Invertebrates in testing of environmental chemicals: are they alternatives?** Environ Health Perspect 1998;106(Suppl 2):593-611.
An enlarged interpretation of alternatives in toxicology testing includes the replacement of one animal species with another, preferably a nonmammalian species. This paper reviews the potential of invertebrates in testing environmental chemicals and provides evidence of their usefulness in alternative testing methodologies. The first part of this review addresses the use of invertebrates in laboratory toxicology testing. Problems in extrapolating results obtained in invertebrates to those obtained from vertebrates are noted, suggesting that invertebrates can essentially be used in addition to rather than as replacements for vertebrates in laboratory toxicity tests. However, evaluation of the ecologic impact of environmental chemicals must include defining end points that may frequently differ from those classically used in biomedical research. In this context, alternative approaches using invertebrates may be more pertinent. The second part of the review therefore focuses on the use of invertebrates in situ to assess the environmental impact of pollutants. Advantages of invertebrates in ecotoxicologic investigation are presented for their usefulness for seeking mechanistic links between effects occurring at the individual level and consequences for higher levels of biologic organization (e.g., population and community). In the end, it is considered that replacement of vertebrates by invertebrates in ecotoxicity testing is likely to become a reality when basic knowledge of metabolic, physiologic, and developmental patterns in the latter will be sufficient to assess the effect of a given

chemical through end points that could be different between invertebrates and vertebrates.

Parchment RE, Gordon M, Grieshaber CK, Sessa C, Volpe D, Ghielmini M. **Predicting hematological toxicity (myelosuppression) of cytotoxic drug therapy from in vitro tests.** Ann Oncol 1998;9(4):357-64.
Several clinical oncology units are studying the roles of in vitro hematotoxicology in phase I evaluations. At the same time, the European Center for the Validation of Alternative Methods (ECVAM) is supporting a validation study of the CFU-GM assay. It is important that these activities be coordinated so that high performance, optimized technical protocols are used for prospective and retrospective clinical evaluations. The EROTC, the NCI and ECVAM could provide support for these coordinated efforts. There is an opportunity for medical oncologists involved in early clinical trials to participate in the evaluation of in vitro tests and their clinical application . Fundamental to acceptance of these assays by oncologists and regulatory scientists, they must predict clinical outcome for myelosuppressive agents and then improve phase I design and performance. These achievements would justify more aggressive dose escalation schemes using guidance from in vitro studies without compromising patient safety. Success in predicting neutropenia might also stimulate the research required to understand how to predict other hematologic toxicities, such as a thrombocytopenia. The complexity of a validation study in hematotoxicology is that it seeks to predict the level of exposure that causes neutropenia, in contrast to other validation studies that have sought to classify a xenobiotic as toxic or not. It may be that the clinical relevance of a new assay is not just a yes-no answer. This important distinction came from the realization that the xenobiotic tolerance in other organ systems of the body must be the same or greater than marrow in order for myelosuppression to be a clinical consequence of exposure. Pharmacological principles of system exposure and toxicity that are integrated into the prediction model provide the links to clinical oncology. It is also important to anticipate future applications of in vitro hematotoxicology. If the maximum tolerated level of drug exposure for human hematopoietic cells can be predicted, then in vitro hematotoxicology could play an important role in new drug discovery. One concept involves screening for compounds that show efficacy at the IC level that predicts maximum tolerated exposure levels in the human. 'Therapeutic index based' drug discovery has been applied to the tallimustine family with some success.

Placke ME. **Development of new candidate drugs. Part 1. In vitro studies.** Appl Clin Trials 1997 Oct;6:36-8.
IPA COPYRIGHT: ASHP An overview of the overall process of preclinical drug development is presented, including strategies and focuses on the decision points and issues that have the greatest impact on efficiency, data quality, and development; in vitro assays that provide the information necessary to plan the first studies in humans, drug formulation and bioanalytical characterization, and information on in vitro cytotoxicity and genotoxicity assays are also examined.

Purchase IF, Botham PA, Bruner LH, Flint OP, Frazier JM, Stokes WS. **Workshop overview: scientific and regulatory challenges for the reduction, refinement, and replacement of animals in toxicity testing.** Toxicol Sci 1998;43(2):86-101.
Public concern for animal welfare has been expressed through legislative control of animal use for experimental purposes since the first legislation was introduced in 1876 in the United Kingdom. Legislative control of animal use has been introduced in virtually every developed country, with major initiatives in Europe (1986) and the United States (1966 and 1985). Advances in scientific thinking resulted in the development of the concept of the three Rs--refinement, reduction, and replacement--by Russell and Burch in 1959. The field has expanded substantially since, with specialist scientific journals dedicated to alternatives, World Congresses organized to discuss the scientific and philosophical issues, and European and U.S. validation organizations being launched. Current scientific attention is focused on validation of alternative methods. The underlying scientific principles of chemical toxicity are complicated and insufficiently understood for alternative methods for all toxicity endpoints of importance in protecting human health to be available. Important lessons have been learned about how to validate methods, including the need to have prediction models available before the validation is undertaken, the need to understand the variability of the animal-based data which is to be used as the validation standard, and the need to have well-managed validation programs. Future progress will depend on the development of novel methods, which can now be validated through international collaborative efforts.

Robinson D. **The International Life Sciences Institute's role in the evaluation of alternative methodologies for the assessment of carcinogenic risk.**Toxicol Pathol 1998;(4):474-5.
BIOSIS COPYRIGHT: BIOL ABS. RRM JOURNAL ARTICLE ANIMAL MOUSE ANIMAL MODEL INTERNATIONAL LIFE SCIENCES INSTITUTE CARCINOGENIC RISK ASSESSMENT HARMONIZATION CARCINOGENICITY TESTING CARCINOGENS PHARMACEUTICALS PROTOCOL STANDARDIZATION TOXICOLOGY PUBLIC HEALTH COMPANY-ORGANIZATION.

Sells PG, Ioannou P, Theakston RD. **A humane alternative to the measurement of the lethal effects (LD50) of non-neurotoxic venoms using hens' eggs.** Toxicon 1998;36(7):985-91.
The accurate measurement of venom lethality is the basis of clinical treatment of snakebite and of much venom-related research. Lethality tests are necessarily carried out in animal models and the results extrapolated to man. While we may be confounded by the obvious limitations of this approach, we can improve the situation by using a non-sentient living system, such as the very early developmental stage of the chick embryo, as an alternative to lethality testing in mammals. The continuing need for lethality testing of venoms and their isolated components, which underpins the development and assessment of antivenoms, currently accounts for thousands of mice annually; this is becoming increasingly unacceptable, first because of the amount of suffering caused and second, because of the high cost incurred. We describe here the use of 4 6 d old chick embryos as a system for estimating venom lethality. The shell-less yolk sac membrane offers a vascular system which develops before intact nervous reflex arcs are functional and therefore the embryo is incapable of experiencing pain. Venom is applied to the membrane on a filter paper disc and its effects on vascular and cardiac function are easily observed throughout the 6 h experiment. Eight venoms tested on eggs and by conventional LD50 assays in mice were compared. A highly significant correlation was obtained suggesting that this simple and inexpensive test would be a far more acceptable alternative for non-neurotoxic venoms.

Spielmann H, Balls M, Dupuis J, Pape WJ, Pechovitch G, De Silva O, Holzhuetter HG, Clothier R, Desolle P, Gerberick F, et al. **The International EU/COLIPA in vitro Phototoxicity Validation Study: Results of phase II (Blind Trial). Part 1. The 3T3 NRU phototoxicity test.** Toxicol In Vitro 1998;12(3):305-27.
BIOSIS COPYRIGHT: BIOL ABS. To date, no standardized international guideline for the testing of chemicals for phototoxic potential has been accepted for regulatory purposes. In 1991, the European Commission (EC), represented initially by the Directorate General XI and later by ECVAM (the European Centre for the Validation of Alternative Methods) and COLIPA (the European Cosmetic, Toiletry and Perfumery Association), agreed to establish a joint EU/COLIPA programme on the development and validation of in vitro phototoxicity tests. The first phase (phase 1, 1992-93) was designed as a prevalidation study, to identify in vitro test procedures and test protocols for a formal validation trial under blind conditions. In the second phase (phase II, 1994-95), the formal validation study, the most promising in vitro phototoxicity tests were validated with 30 carefully selected test chemicals in 11 laboratories in a blind trial. The 3T3 mouse fibroblast neural red uptake phototoxicity test (3T3 NRU PT) was performed as a core test in nine laboratories, slice it provided the best results in phase I of the study. The purpose of phase II was to confirm the reliability and relevance of the in vitro tests for predicting phototoxic effects and for identifying phototoxic chemicals. In phase II the phototoxic potential of test chemicals in the 3T3 NRU PT test was either assessed by determining the phototoxicity factor (PIF) by using a cut-off value of 5 as in phase I of the study, or by determining the mean photo effect (MPE) by using a cut-off value of 0.1, as recently proposed by Holzhutter (1997). Results obtained with both approaches in the 3T3 NRU PT test in phase II were reproducible in the nine laboratories, and the correlation between in vitro and in vivo data was very high. Therefore, ECVAM and COLIPA conclude from this formal validation trial under blind conditions that the 3T3 NRU Pt test is a scientifically validated in vitro test which is ready to be considered for regulatory purposes for assessing the phototoxic potential of chemicals. A draft OECD Guideline for "In Vitro Phototoxicity Testing", incorporating the standard protocol of the 3T3 NRU PT test, will be submitted to the OECD test guidelines programme in due course.

Spielmann H, Bochkov NP, Costa L, Gribaldo L, Guillouzo A, Heindel JJ, Karol M, Parchment R, Pfaller W, Peraita PP, et al. **13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals**

(SGOMSEC): alternative testing methodologies for organ toxicity. Environ Health Perspect 1998;106(Suppl 2):427-39.
In the past decade in vitro tests have been developed that represent a range of anatomic structure from perfused whole organs to subcellular fractions. To assess the use of in vitro tests for toxicity testing, we describe and evaluate the current status of organotypic cultures for the major target organs of toxic agents. This includes liver, kidney, neural tissue, the hematopoietic system, the immune system, reproductive organs, and the endocrine system. The second part of this report reviews the application of in vitro culture systems to organ specific toxicity and evaluates the application of these systems both in industry for safety assessment and in government for regulatory purposes. Members of the working group (WG) felt that access to high-quality human material is essential for better use of in vitro organ and tissue cultures in the risk assessment process. Therefore, research should focus on improving culture techniques that will allow better preservation of human material. The WG felt that it is also important to develop and make available relevant reference compounds for toxicity assessment in each organ system, to organize and make available via the Internet complete in vivo toxicity data, including human data, containing dose, end points, and toxicokinetics. The WG also recommended that research should be supported to identify and to validate biological end points for target organ toxicity to be used in alternative toxicity testing strategies.

Stokes WS, Marafante E. **Introduction and summary of the 13th meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): alternative testing methodologies.** Environ Health Perspect 1998;106(Suppl 2):405-12.
A workshop on alternative toxicological testing methodologies was convened by the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) 26-31 January 1997 in Ispra, Italy, at the European Centre for the Validation of Alternative Methods. The purpose of the workshop was to assess the current status of alternative testing methodologies available to evaluate adverse human health and environmental effects of chemicals. Another objective of the workshop was to identify and recommend research needed to fill knowledge gaps that would lead to new test methodologies. Four work groups were established to address conceptual issues, acute toxicity, organ toxicity, and ecotoxicology. A joint workshop report was prepared for each topic and included recommendations for the development and use of alternative methods. Participants concluded that alternative methods and approaches are available that can be incorporated into tiered strategies for toxicological assessments. Use of these methods will reduce the numbers of animals required, and in some instances reduce animal pain and distress. It was recommended that future efforts to develop test methods should emphasize mechanism-based methods that can provide improved predictions of toxicity. Continued international cooperation was encouraged to facilitate future progress in the development of alternative toxicological testing methods. These methods will provide for improvements in human health protection, environmental protection, and animal welfare.

Walker CH. **Biomarker strategies to evaluate the environmental effects of chemicals.** Environ Health Perspect 1998;106(Suppl 2):613-20.
Environmental risk assessment of chemicals depends on the production of toxicity data for surrogate species of mammals, birds, and fish and on making comparisons between these and estimated or predicted environmental concentrations of the chemicals. This paper gives an overview of biomarker assays and strategies that might be used as alternatives, that is, to replace, reduce, or refine currently used ecotoxicity tests that cause suffering to vertebrates. In the present context a biomarker is a biologic response to an environmental chemical at the individual level or below which demonstrates a departure from normal status. Of immediate interest and relevance are nondestructive assays that provide a measure of toxic effect in vertebrate species and that can be used in both laboratory and parallel field studies. A major shortcoming of this approach is that such assays are currently only available for a limited number of chemicals, primarily when the mode of action is known. Nondestructive assays can be performed on blood, skin, excreta, and eggs of birds, fish, reptiles, and amphibians. An interesting recent development is the use of vertebrate cell cultures, including transgenic cell lines that have been developed specifically for toxicity testing. The ultimate concern in ecotoxicology is the effects of chemicals at the level of populations and above. Current risk assessment practices do not address this problem. The development of biomarker strategies could be part of a movement toward more ecologic end points in the safety evaluation of chemicals, which would effect a reduction in animal tests that cause suffering.

Walker C, Kaiser K, Klein W, Lagadic L, Peakall D, Sheffield S, Soldan T, Yasuno M. **13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): alternative testing methodologies for ecotoxicity.** Environ Health Perspect 1998;106(Suppl 2):441-51.
There is growing public pressure to minimize the use of vertebrates in ecotoxicity testing; therefore, effective alternatives to toxicity tests causing suffering are being sought. This report discusses alternatives and differs in some respects from the reports of the other three groups because the primary concern is with harmful effects of chemicals at the level of population and above rather than with harmful effects upon individuals. It is concluded that progress toward the objective of minimizing testing that causes suffering would be served by the following initiatives--a clearer definition of goals and strategies when undertaking testing procedures; development of alternative assays, including in vitro test systems, that are based on new technology; development of nondestructive assays for vertebrates (e.g., biomarkers) that do not cause suffering; selection of most appropriate species, strains, and developmental stages for testing procedures (but no additional species for basic testing); better integrated and more flexible testing procedures incorporating biomarker responses, ecophysiological concepts, and ecological end points (progress in this direction depends upon expert judgment). In general, testing procedures could be made more realistic, taking into account problems with mixtures, and with volatile or insoluble chemicals.

Walum E. **Acute oral toxicity.** Environ Health Perspect 1998;106(Suppl 2):497-503.
The purposes of acute toxicity testing are to obtain information on the biologic activity of a chemical and gain insight into its mechanism of action. The information on acute systemic toxicity generated by the test is used in hazard identification and risk management in the context of production, handling, and use of chemicals. The LD50 value, defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in a given period, is currently the basis for toxicologic classification of chemicals. For a classical LD50 study, laboratory mice and rats are the species typically selected. Often both sexes must be used for regulatory purposes. When oral administration is combined with parenteral, information on the bioavailability of the tested compound is obtained. The result of the extensive discussions on the significance of the LD50 value and the concomitant development of alternative procedures is that authorities today do not usually demand classical LD50 tests involving a large number of animals. The limit test, the fixed-dose procedure, the toxic class method, and the up-and-down methods all represent simplified alternatives using only a few animals. Efforts have also been made to develop in vitro systems; e.g., it has been suggested that acute systemic toxicity can be broken down into a number of biokinetic, cellular, and molecular elements, each of which can be identified and quantified in appropriate models. The various elements may then be used in different combinations to model large numbers of toxic events to predict hazard and classify compounds.

Zeiger E, Stokes WS. **Validating new toxicology tests for regulatory acceptance.** Regul Toxicol Pharmacol 1998;27(1 Pt 1):32-7.
Before a new or revised toxicology test is considered acceptable for safety evaluation of new substances, the test users and the industrial and regulatory decision makers must feel comfortable with it, and the decisions it supports. Comfort with, and the acceptance of, a new test comes after knowing that it has been validated for its proposed use. The validation process is designed to determine the operational characteristics of a test, that is, its reliability and relevance, in addition to its strengths and limitations. The reliability of a test is measured by its reproducibility. Its relevance is judged by its mechanistic relationship to the health effects of concern, and its ability to predict or identify those effects. The U.S. government has recently formed the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to work with federal agencies and test developers to coordinate the evaluation and adoption of new test methods. The ICCVAM will provide guidance to agencies and other stakeholders on criteria and processes for development, validation, and acceptance of tests; coordinate technical reviews of proposed new tests of interagency interest; facilitate information sharing among agencies; and serve as an interagency resource and communications link with parties outside of the federal government on matters of test method validation.

CARCINOGENESIS

Akimoto T, Mitsuhashi N, Saito Y, Ebara T, Niibe H. **Effect of radiation on the expression of E-cadherin and alpha-catenin and invasive capacity in human lung cancer cell line in vitro.** Int J Radiat Oncol Biol Phys 1998;41(5):1171-6.
PURPOSE: To investigate the effect of radiation on E-cadherin and alpha-catenin expression in a human lung cancer cell line, and also evaluate invasive capacity in the membrane invasion culture system using the Boyden Chamber. MATERIALS AND METHODS: The immunoblot and immunofluorescence analyses were performed using the human lung cancer cell line A549 to examine altered expression of E-cadherin and alpha-catenin after irradiation. We also compared invasive capacity of untreated cells with that of irradiated cells. RESULTS: Immunoblot analysis revealed that the expression of E-cadherin increased after irradiation. In a time-course analysis, the expression was increased 6 h after irradiation with 10 Gy and reached its peak level at 24 h, being 2.3 times the control value, whereas expression at 1 and 3 h after irradiation was almost equivalent to that of the control. A slight increase in expression was observed after irradiation of 2 Gy and the expression reached peak levels after 5 Gy. After fractionated irradiation, the increase in expression of both E-cadherin and alpha-catenin was observed, and the alteration of alpha-catenin was more prominent than that after a single irradiation of the same total dose. In the immunofluorescence study for E-cadherin antibody analyzed by confocal laser scanning microscopy, increased intensity in irradiated cells produced as a nondisrupted and continuous line at cell-cell contact sites. In an invasive assay, the number of migrated cells in irradiated cells after a dose of 5 and 10 Gy was reduced significantly compared to untreated cells. CONCLUSION: The results indicate that irradiation of A549 increased the expression of E-cadherin, possibly preserving their functional property.

Azin F, Raie RM, Mahmoudi MM. **Correlation between the levels of certain carcinogenic and anticarcinogenic trace elements and esophageal cancer in northern Iran.** Ecotoxicol Environ Saf 1998;39(3):179-84.
Levels of four carcinogenic (Ni, Fe, Cu, Pb) and four anticarcinogenic (Zn, Se, Mn, Mg) trace elements were measured in hair samples from esophageal cancer patients, their unaffected family members, and members of families with no history of cancer. Measurements were also made in non-esophageal cancer patients, using atomic absorption spectroscopy, inductively coupled plasma-emission spectroscopy, and neutron activation analysis. The results showed that Ni and Cu concentrations were significantly higher and Mg and Mn concentrations were significantly lower in all cancer cases. Levels of Zn, Fe, Se, and Pb were not significantly different in the above-mentioned groups. In addition, the serum albumin fraction, which is reported to have antioxidant activity, was found to be significantly lower among esophageal cancer patients.

Balanchandra Dass S, Heflich RH, Casciano DA. **Mutational response at the splenic T-lymphocyte hprt locus in mice treated as neonates: Contrasting effects of the carcinogens N-ethyl-N-nitrosourea, dimethylnitrosamine, and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine.** Environ Mol Mutagen 1998;31 (3):243-7.
BIOSIS COPYRIGHT: BIOL ABS. The newborn mouse tumorigenicity assay, which involves the treatment of animals during the first two weeks after birth and monitoring tumor induction after a year, has been suggested as a cost- and time-effective alternative to the conventional two year rodent bioassay. In order to evaluate whether or not lymphocyte hprt mutant induction is an accurate predictor of carcinogenicity in the assay, we determined the Frequencies of 6-thioguanine-resistant (TGr) lymphocytes in the spleens of mice neonatally treated with the carcinogenic mutagens N-ethyl-N-nitrosourea (ENU), dimethylnitrosamine (DMN), and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP). Male C57BL/6 pups were injected on postnatal days 8 and 15, and the frequency of TGr T-lymphocytes was measured in groups of three animals, sacrificed periodically up to 31 weeks posttreatment. Compared to background frequencies of 1.1 -2.9ant frequencies (MFS) reached 155.1ve dose of 49 mg ENU/kg body weight and 172.3 a cumulative dose of 142 mg ENU/kg. These results show that TGr lymphocyte mutations can be induced and measured in mice treated as neonates and that the induced MFs found for mice treated neonatally with ENU are comparable with frequencies reported for the treatment of adult animals with the same chemical. In contrast, treatment with the promutagenic and procarcinogenic compounds DMN (at a maximum concentration of 10.5 mg/kg) and PhIP (26.2 mg/kg) did not result in an increase in lymphocyte MF, suggesting that

reactive metabolites of these compounds may not be reaching cells that are sensitive for mutation fixation. The results indicate that the lymphocyte hprt assay may fail to predict the carcinogenicity of some test chemicals in the neonatal mouse bioassay.

Barbin A. **Formation of DNA etheno adducts in rodents and humans and their role in carcinogenesis.** Acta BioChim Pol 1998;45(1):145-61.
BIOSIS COPYRIGHT: BIOL ABS. Ethenobases are exocyclic adducts formed with DNA by some environmental carcinogens such as vinyl chloride or urethane. In the last few years, they have received a renewed interest due to the development of sensitive techniques of analysis that made it possible to measure their formation in vivo. This minireview summarizes the information gained recently from the work of several laboratories, including ours. Increased levels of DNA etheno adducts have been measured in target tissues from rodents exposed to vinyl chloride or urethane. Hepatic tumours caused by exposure to vinyl chloride in humans and in rats and lung tumours induced by urethane in mice exhibit base pair substitution mutations in the ras and p53 genes which seem to be exposure specific and consistent with the promutagenic properties of ethenobases. Background levels of etheno adducts have been detected in DNA from non-exposed humans or animals, pointing to an alternative, endogenous pathway of formation. This background may be affected by dietary factors. It could arise from the reaction of trans-4-hydroxy-2-nonenal (or its epoxide 2,3-epoxy-4-hydroxynonanal), a lipid peroxidation product, with nucleic acid bases. Elevated levels of etheno adducts are found in hepatic DNA from humans and rodents with genetic predisposition to oxidative stress and lipid peroxidation in the liver, and with an associated increased risk of liver cancer. These data suggest that DNA ethenobases could serve as new biomarkers of oxidative stress/lipid peroxidation.

Beland FA, Schmitt TC, Fullerton NF, Young JF. **Metabolism of chloral hydrate in mice and rats after single and multiple doses.** J Toxicol Environ Health 1998;54(3):209-26.
Chloral hydrate is a hepatocarcinogen in mice but not rats. To examine this species-related difference, male and female B6C3F1 mice and Fischer (F344) rats were treated by gavage with 1 or 12 doses of chloral hydrate, and concentrations of the drug and its metabolites were determined in plasma at 0.25, 7, 3, 6, and 24 h and 2, 4, 8, and 16 d after the last treatment. Maximum levels of chloral hydrate were observed at the initial sampling time of 0.25 h. By 1 h, levels dropped substantially, and by 3 h, chloral hydrate could not be detected. Trichloroacetic acid was the major metabolite found in the plasma, with peak levels being observed 1-6 h after dosing. The concentrations then slowly decreased such that by 2 d this metabolite could no longer be detected. Trichloroethanol was assayed as both the free alcohol and its glucuronide. Maximum levels of trichoroethanol occurred at 0.25 h, and by 1-3 h approached the limits of detection. A pharmacokinetic model was constructed to describe the metabolic data. The plasma half-life values of chloral hydrate were similar in both species. In mice, the rate of elimination of trichloroacetic acid was significantly increased after multiple doses; this difference was not observed with rats. The half-life of trichloroethanol and its glucuronide was significantly greater in rats as compared to mice. None of the metabolic parameters appears to account for the hepatocarcinogenicity of chloral hydrate seen in mice but not rats.

Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Saccomanno G, Gabrielson E, Baylin SB, Herman JG. **Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis.** Proc Natl Acad Sci U S A 1998;95(20):11891-6.
The p16(INK4a) (p16) tumor suppressor gene can be inactivated by promoter region hypermethylation in many tumor types including lung cancer, the leading cause of cancer-related deaths in the U.S. We have determined the timing of this event in an animal model of lung carcinogenesis and in human squamous cell carcinomas (SCCs). In the rat, 94% of adenocarcinomas induced by the tobacco specific carcinogen 4-methylnitrosamino-1-(3-pyridyl)-1-butanone were hypermethylated at the p16 gene promoter; most important, this methylation change was frequently detected in precursor lesions to the tumors: adenomas, and hyperplastic lesions. The timing for p16 methylation was recapitulated in human SCCs where the p16 gene was coordinately methylated in 75% of carcinoma in situ lesions adjacent to SCCs harboring this change. Moreover, the frequency of this event increased during disease progression from basal cell hyperplasia (17%) to squamous metaplasia (24%) to carcinoma in situ (50%) lesions. Methylation of p16 was associated with loss of expression in both tumors and precursor lesions indicating that both

alleles were functionally inactivated. The potential of using assays for aberrant p16 methylation to identify disease and/or risk was validated by detection of this change in sputum from three of seven patients with cancer and 5 of 26 cancer-free individuals at high risk. These studies show for the first time that an epigenetic alteration, aberrant methylation of the p16 gene, can be an early event in lung cancer and may constitute a new biomarker for early detection and monitoring of prevention trials.

Beyer U, Roth T, Schumacher P, Maier G, Unold A, Frahm AW, Fiebig HH, Unger C, Kratz F. **Synthesis and in vitro efficacy of transferrin conjugates of the anticancer drug chlorambucil.** J Med Chem 1998;41(15):2701-8.
One strategy for improving the selectivity and toxicity profile of antitumor agents is to design drug carrier systems employing soluble macromolecules or carrier proteins. Thus, five maleimide derivatives of chlorambucil were bound to thiolated human serum transferrin which differ in the stability of the chemical link between drug and spacer. The maleimide ester derivatives 1 and 2 were prepared by reacting 2-hydroxyethylmaleimide or 3-maleimidophenol with the carboxyl group of chlorambucil, and the carboxylic hydrazone derivatives 5-7 were obtained through reaction of 2-maleimidoacetaldehyde, 3-maleimidoacetophenone, or 3-maleimidobenzaldehyde with the carboxylic acid hydrazide derivative of chlorambucil. The alkylating activity of transferrin-bound chlorambucil was determined with the aid of 4-(4-nitrobenzyl)pyridine (NBP) demonstrating that on average 3 equivalents were protein-bound. Evaluation of the cytotoxicity of free chlorambucil and the respective transferrin conjugates in the MCF7 mammary carcinoma and MOLT4 leukemia cell line employing a propidium iodide fluorescence assay demonstrated that the conjugates in which chlorambucil was bound to transferrin through non-acid-sensitive linkers, i.e., an ester or benzaldehyde carboxylic hydrazone bond, were not, on the whole, as active as chlorambucil. In contrast, the two conjugates in which chlorambucil was bound to transferrin through acid-sensitive carboxylic hydrazone bonds were as active as or more active than chlorambucil in both cell lines. Especially, the conjugate in which chlorambucil was bound to transferrin through an acetaldehyde carboxylic hydrazone bond exhibited IC50 values which were approximately 3-18-fold lower than those of chlorambucil. Preliminary toxicity studies in mice showed that this conjugate can be administered at higher doses in comparison to unbound chlorambucil. The structure-activity relationships of the transferrin conjugates are discussed with respect to their pH-dependent acid sensitivity, their serum stability, and their cytotoxicity.

Carter S, Auer KL, Reardon DB, Birrer M, Fisher PB, Valerie K, Schmidt-Ullrich R, Mikkelsen R, Dent P. **Inhibition of the mitogen activated protein (MAP) kinase cascade potentiates cell killing by low dose ionizing radiation in A431 human squamous carcinoma cells.** Oncogene 1998;16(21):2787-96.
The molecular mechanism(s) by which tumor cells survive after exposure to ionizing radiation are not fully understood. Exposure of A431 cells to low doses of radiation (1 Gy) caused prolonged activations of the mitogen activated protein (MAP) kinase and stress activated protein (SAP) kinase pathways, and induced p21(Cip-1/WAF1) via a MAP kinase dependent mechanism. In contrast, higher doses of radiation (6 Gy) caused a much weaker activation of the MAP kinase cascade, but a similar degree of SAP kinase cascade activation. In the presence of MAP kinase blockade by the specific MEK1 inhibitor (PD98059) the basal activity of the SAP kinase pathway was enhanced twofold, and the ability of a 1 Gy radiation exposure to activate the SAP kinase pathway was increased approximately sixfold 60 min after irradiation. In the presence of MAP kinase blockade by PD98059 the ability of a single 1 Gy exposure to cause double stranded DNA breaks (TUNEL assay) was enhanced at least threefold over the following 24-48 h. The increase in DNA damage within 48 h was also mirrored by a similar decrease in A431 cell growth as judged by MTT assays over the next 4-8 days following radiation exposure. This report demonstrates that the MAP kinase cascade is a key cytoprotective pathway in A431 human squamous carcinoma cells which is activated in response to clinically used doses of ionizing radiation. Inhibition of this pathway potentiates the ability of low dose radiation exposure to induce cell death in vitro.

Cavin C, Holzhauser D, Constable A, Huggett AC, Schilter B. **The coffee-specific diterpenes cafestol and kahweol protect against aflatoxin B1-induced genotoxicity through a dual mechanism.** Carcinogenesis 1998;19(8):1369-75.
The diterpenes cafestol and kahweol (C&K) have been identified in animal models as two potentially chemoprotective agents present in green and roasted coffee beans. It has been postulated that these compounds

may act as blocking agents by producing a co-ordinated modulation of multiple enzymes involved in carcinogen detoxification. In this study, we investigated the effects of C&K against the covalent binding of aflatoxin B1 (AFB1) metabolites to DNA. Male Sprague-Dawley rats were treated with increasing amounts of a mixture of C&K in the diet (0-6200 p.p.m.) for 28 and 90 days. A dose-dependent inhibition of AFB1 DNA-binding was observed using S9 and microsomal subcellular fractions from C&K-treated rat liver in an in vitro binding assay. Significant inhibition was detected at 2300 p.p.m. and maximal reduction of DNA adduct formation to nearly 50% of the control value was achieved with 6200 p.p.m. of dietary C&K. Two complementary mechanisms may account for the chemopreventive action of cafestol and kahweol against aflatoxin B1 in rats. A decrease in the expression of the rat activating cytochrome P450s (CYP2C11 and CYP3A2) was observed, as well as a strong induction of the expression of the glutathione-S-transferase (GST) subunit GST Yc2, which is known to detoxify highly the most genotoxic metabolite of AFB1. These data and the previously demonstrated effects of C&K against the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis at various tissue sites suggest the potential widespread effect of these coffee components against chemical carcinogenesis.

Chandler LA, Sosnowski BA, McDonald JR, Price JE, Aukerman SL, Baird A, Pierce GF, Houston LL. **Targeting tumor cells via EGF receptors: selective toxicity of an HBEGF-toxin fusion protein.** Int J Cancer 1998;78 (1):106-11.
Over-expression of the epidermal growth factor receptor (EGFR) is a hallmark of numerous solid tumors, thus providing a means of selectively targeting therapeutic agents. Heparin-binding epidermal growth factor (HBEGF) binds to EGFRs with high affinity and to heparan sulfate proteoglycans, resulting in increased mitogenic potential compared to other EGF family members. We have investigated the feasibility of using HBEGF to selectively deliver a cytotoxic protein into EGFR-expressing tumor cells. Recombinant fusion proteins consisting of mature human HBEGF fused to the plant ribosome-inactivating protein saporin (SAP) were expressed in Escherichia coli. Purified HBEGF-SAP chimeras inhibited protein synthesis in a cell-free assay and competed with EGF for binding to receptors on intact cells. A construct with a 22-amino-acid flexible linker (L22) between the HBEGF and SAP moieties exhibited an affinity for the EGFR that was comparable to that of HBEGF. The sensitivity to HBEGF-L22-SAP was determined for a variety of human tumor cell lines, including the 60 cell lines comprising the National Cancer Institute Anticancer Drug Screen. HBEGF-L22-SAP was cytotoxic in vitro to a variety of EGFR-bearing cell lines and inhibited growth of EGFR-over-expressing human breast carcinoma cells in vivo. In contrast, the fusion protein had no effect on small-cell lung carcinoma cells, which are EGFR-deficient. Our results demonstrate that fusion proteins composed of HBEGF and SAP exhibit targeting specificity and cytotoxicity that may be of therapeutic value in treating a variety of EGFR-bearing malignancies.

Chevillard S, Radicella JP, Levalois C, Lebeau J, Poupon MF, Oudard S, Dutrillaux B, Boiteux S. **Mutations in OGG1, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours.** Oncogene 1998;16(23):3083-6. The human OGG1 gene encodes a DNA glycosylase activity catalysing the excision of the mutagenic lesion 7,8-dihydro-8-oxoguanine from oxidatively damaged DNA. The OGG1 gene was localized to chromosome 3p25, a region showing frequent loss of heterozygosity (LOH) in lung and kidney tumours. In this study, we have analysed by RT-PCR the expression of OGG1 in 25 small cell lung cancers, in 15 kidney carcinomas and the 15 normal kidney counterparts. The results show that OGG1 messenger RNA can be detected in all tumours tested and that no significant difference was observed in the level of expression between normal and tumoral kidney tissues. Denaturing gradient gel electrophoresis (DGGE) was used to screen this series of human tumours for alterations in the OGG1 cDNA. The study revealed homozygous mutations in three tumours, two from lung and one from kidney. Sequencing analysis of the mutants identified a single base substitution in each of the three cases: two transversions (GC to TA and TA to AT) and one transition (GC to AT). All three substitutions cause an amino acid change in the hOgg1 protein. For the mutant kidney tumour, the normal tissue counterpart shows a wild-type profile. These results suggest a role for OGG1 mutations in the course of the multistage process of carcinogenesis in lung or kidney.

Cunningham AR, Klopman G, Rosenkranz HS. **Identification of structural features and associated mechanisms of action for carcinogens in rats.** Mutat Res 1998;405(1):9-27.

A set of chemicals tested for carcinogenicity in rats that have been analyzed in the Carcinogenic Potency Database (CPDB) was subjected to CASE/MULTICASE (a computer-automated structure evaluation system) structure-activity relationship (SAR) analyses. This SAR system identifies structural features of chemicals in a learning set that are associated with a predefined activity and produces an SAR model based on these characteristics. The rat CPDB used in this study consisted of 745 chemicals, 383 of which are carcinogens, 14 marginally active carcinogens (i.e., chemicals that require a relatively high dose to induce carcinogenesis) and 348 are non-carcinogens. In an internal prediction analysis where CASE/MULTICASE 'predicted' the activity of chemicals in the learning set, the system was able to achieve a concordance between experimental and predicted results of 95%. This indicates that the program is able to adequately assess the chemicals in the database. In a 10-fold cross-validation study where 10 disjoint sets of 10% of the chemicals were removed from the database and the remaining 90% of the chemicals were used as a learning set, CASE/MULTICASE was able to achieve a concordance between experimental and predicted results of 64%. Using a modified validation process designed to investigate the predictivity of a more focused SAR model, the system was able to achieve a concordance of 71% between experimental and predicted results. Among the major biophores identified by CASE/MULTICASE as associated with cancer causation in rats, several are derived from electrophilic or potentially electrophilic compounds (e.g., aromatic amines, nitrogen mustards, isocyanates, epoxides). Other biophores however are derived from chemicals seemingly devoid of actual or potential DNA-reactivity and as such may represent structural features of non-genotoxic carcinogens. Copyright 1998 Elsevier Science B.V.

Dal Cin P, Timmerman D, Van Den Berghe I, Wanschura S, Kazmierczak B, Vergote I, Deprest J, Neven P, Moerman P, Bullerdiek J, et al. **Genomic changes in endometrial polyps associated with tamoxifen show no evidence for its action as an external carcinogen.** Cancer Res 1998;58(11):2278-81.
Eighty-eight endometrial specimens from 36 postmenopausal breast cancer patients treated with tamoxifen were investigated cytogenetically and molecularly using fluorescence in situ hybridization with appropriate probes for the HMGIC and HMGIY genes. Twenty control specimens, 10 endometrial polyps, and 10 endometrial biopsy specimens were investigated in the same way. Of the 88 specimens, 44 were from endometrial polyps; 3 were from endocervical polyps; 7 were from cystic endometrium; 30 were from normal or atrophic endometrium, normal endocervix, or myometrium; and 4 were from endometrial carcinomas. Chromosome investigation of the endometrial polyps showed the nature of the chromosome changes in tamoxifen-induced polyps to be the same as that in the controls and in sporadic endometrial polyps described in the literature. HMGIC and HMGIY gene rearrangements in both groups were identical as shown by fluorescence in situ hybridization, which also allowed for the detection of seven hidden paracentric inversions involving 12q15, one of which occurred in a cystic endometrium. The carcinomas did not exhibit any of these changes. Because abnormal expression of HMGIC or HMGIY as a consequence of structural chromosome changes in 12q15 or 6p21, respectively, is invariably associated with benign neoplasia, tamoxifen-associated endometrial polyps are unlikely to undergo further malignant transformation, and a mode of action of tamoxifen as an external carcinogen is unlikely.

De Filippo C, Caderni G, Bazzicalupo M, Briani C, Giannini A, Fazi M, Dolara P. **Mutations of the Apc gene in experimental colorectal carcinogenesis induced by azoxymethane in F344 rats.** Br J Cancer 1998;77 (12):2148-51.
BIOSIS COPYRIGHT: BIOL ABS. We investigated in the rat the role of the Apc gene, which is mutated in familial adenomatous polyposis and sporadic colon cancer in the process leading from normal colonic mucosa to aberrant crypt foci (ACF) and finally to adenomas and adenocarcinomas. We analysed mutations in exon 15 of the rat Apc gene using in vitro synthesized protein assay in 66 ACF and in 28 colon tumours induced by azoxymethane. No Apc mutations were found in ACF, whereas five mutations were found in the tumours. The data suggest that mutations of the Apc gene are associated with the transition from ACF to adenoma and adenocarcinoma and not from normal mucosa to ACF.

Depenbrock H, Peter R, Faircloth GT, Manzanares I, Jimeno J, Hanauske AR. **In vitro activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells.** Br J Cancer 1998;78(6):739-44.

Aplidine is a new marine anti-cancer depsipeptide isolated from the Mediterranean tunicate Aplidium albicans. We have evaluated its antiproliferative action against a variety of freshly explanted human tumour specimens. Concentration ranges of 0.01-1.0 microM and 0.0001-1.0 microM were used in short- and long-term exposure schedules respectively. After exposure for 1 h in 49 evaluable specimens, aplidine showed a clear concentration-dependent anti-tumour effect. At 0.05 microM, 85% of the specimens were markedly inhibited. Continuous exposure for 21-28 days in 54 tumour specimens also led to a concentration-dependent activity relationship. Fifty per cent and 100% tumour inhibitions were achieved with 0.001 microM and 0.05 microM respectively. A head to head evaluation assessing short vs continuous exposure was carried out, resulting in evidence of an activity-time of exposure relationship. Breast, melanoma and non-small-cell lung cancer appear to be sensitive to low concentrations of aplidine. In addition the evaluation of the effects of aplidine on haematopoietic cells showed a concentration-dependent toxicity. However, under continuous exposure, active concentrations induced mild bone marrow toxicity, indicating that a therapeutic window at marginally myelotoxic concentrations might exist.

Fan S, Wang JA, Yuan RQ, Rockwell S, Andres J, Zlatapolskiy A, Goldberg ID, Rosen EM. **Scatter factor protects epithelial and carcinoma cells against apoptosis induced by DNA-damaging agents.** Oncogene 1998;17 (2):131-41.
Scatter factor (SF) (hepatocyte growth factor) is a cytokine that may play a role in human breast cancer invasiveness and angiogenesis. We now report that SF can block the induction of apoptosis by various DNA damaging-agents, including cytotoxic agents used in breast cancer therapy. SF protected MDA-MB-453 human breast cancer cells, EMT6 mouse mammary tumor cells and MDCK renal epithelial cells against apoptosis induced by adriamycin (ADR), X-rays, ultraviolet radiation, and other agents. Protection was observed in assays of DNA fragmentation, cell viability (MTT), and clonogenic survival. Protection of MDA-MB-453 cells against ADR was dose- and time-dependent; maximal protection required pre-incubation with 75-100 ng/ml of SF for 48 h or more. Protection required functional SF receptor (c-Met), but was not dependent on p53. Western blotting analysis revealed that pre-treatment of MDA-MB-453 cells with SF inhibited the ADR-induced decreases in the levels of Bcl-XL, an anti-apoptotic protein related to Bcl-2; and the dose-response and time course characteristics for SF-mediated increases in the Bcl-XL protein levels of ADR-treated cells were consistent with the degrees of protection against apoptosis observed under the same conditions. Furthermore, Bcl-XL levels were not down-regulated by ADR in MDA-MB-231 breast cancer cells, consistent with the finding that SF failed to protect these cells against ADR, despite the fact that they contain functional c-Met receptor. In contrast to Bcl-XL, SF blocked ADR-induced increases in c-Myc and inhibited the expression of p21WAF1/CIP1 and of the BRCA1 protein in MDA-MB-453 cells. However, SF did not cause significant changes in the cell cycle distribution of ADR-treated cells. These findings suggest that SF-mediated protection of human breast cancer cells may involve inhibition of one or more pathways required for the activation of apoptosis and may particularly target the anti-apoptotic mitochondrial membrane pore-forming protein Bcl-XL as a component of the protective mechanism. By implication, the accumulation of SF within human breast cancers may contribute to the development of a radio- or chemoresistant phenotype.

Fan W, Johnson KR, Miller MC 3rd. **In vitro evaluation of combination chemotherapy against human tumor cells (Review).** Oncol Rep 1998;5(5):1035-42.
Combination therapy with multiple drugs or with multiple modalities is common practice in the treatment of cancer. The purpose of using drugs in combinations is to increase the therapeutic efficacy, decrease toxicity toward the host and minimize or delay the development of drug resistance. Presently used clinical protocols for cancer combination therapy are mainly obtained empirically or from clinical trials. Accumulation of experience from clinical trials is invaluable but is a slow and expensive process. Also, due to heterogeneous patient populations exposed to different environments, human clinical data frequently cannot be used for quantitative synergy determinations. Therefore, in vitro quantitative drug combination studies with cultured tumor cells are becoming imperative either as prospective studies or as adjuvant assessment for combination therapy. In recent years, a variety of in vitro assays have been developed to examine cytotoxicity or biochemical effects of drugs on cultured tumor cells. These methods can, not only quickly predict the potential therapeutic effects of the combined agents, but also provide the information or clues to the possible mechanisms of drug interactions. In addition, with the better understanding of various antineoplastic drugs and the development of new technologies to characterize actions of the drugs, the in

vitro study of combination therapy is no longer limited to the measurement of cytotoxic effects. Instead, many other studies, such as cell cycle analyses, detection of apoptosis and biochemical analyses of drug interactions have also become common methods for the in vitro evaluation of combination drug therapy.

Ferdous AJ, Stembridge NY, Singh M. **Role of monensin PLGA polymer nanoparticles and liposomes as potentiator of ricin A immunotoxins in vitro.** J Controlled Release 1998 Jan 2;50:71-8.
IPA COPYRIGHT: ASHP The preparation and in vitro immunotoxic activity of monensin encapsulated into nanoparticles by using biodegradable polyglactin 370 (poly(DL-lactide-co-glycolide)) are described. The average size of nanoparticles could be decreased from 567 nm to 163 nm by increasing the concentration of polyvinyl alcohol from 10% to 100% of polyglactin 370. The nanoparticles were spherical in shape. The concentration of monensin was more than 12%. The zeta potential of the nanoparticles was -25.8 mv, which did not change significantly after resuspension of the freeze dried sample. The nanoparticles were tested against HL-60 and HT-29 human tumor cell lines in vitro. Monensin nanoparticles potentiated the activity of ricin A based immunotoxins by 40-50 times against these cell lines. There was, however, no differences between the nanoparticles and liposomes for their potentiating effect of immunotoxins against the 2 tumor cell lines.

Gallagher BC, Rudolph DB, Hinton BT, Hanigan MH. **Differential induction of gamma-glutamyl transpeptidase in primary cultures of rat and mouse hepatocytes parallels induction during hepatocarcinogenesis.** Carcinogenesis 1998;19(7):1251-5.
In carcinogen-treated rats, gamma-glutamyl transpeptidase (GGT) is induced in preneoplastic liver lesions and liver tumors. However, in mice, GGT is rarely detected during hepatocarcinogenesis. Data in this study reveal that GGT is not induced in mouse hepatocytes when they are maintained in vitro under the same conditions that induce GGT activity in primary cultures of rat hepatocytes. GGT activity in rat hepatocytes increased 20-fold during the first 7 days in culture, but there was no induction of GGT in primary cultures of mouse hepatocytes. Comparison of intracellular glutathione levels in rat and mouse liver cells showed that the glutathione level was higher in the mouse liver cells than the rat. Blocking glutathione synthesis with buthionine sulfoximine reduced the intracellular glutathione concentration in mouse liver cells but did not trigger an induction of GGT. Analysis of the GGT mRNA in primary cultures of rat hepatocytes showed that only GGT mRNA(III) is induced. This is the same GGT mRNA species present in preneoplastic hepatic lesions and liver tumors in the rat (1-3). Therefore activation of promoter III in the GGT gene is responsible for induction of GGT in both hepatocytes in vitro and liver tumors in vivo. These data show that primary cultures of rat and mouse hepatocytes provide a model system with which to study interspecies differences in the regulation of this enzyme and to better understand the role of GGT in normal and neoplastic processes.

Ghielmini M, Colli E, Bosshard G, Pennella G, Geroni C, Torri V, D'incalci M, Cavalli F, Sessa C. **Hematotoxicity on human bone marrow- and umbilical cord blood-derived progenitor cells and in vitro therapeutic index of methoxymorpholinyldoxorubicin and its metabolites.** Cancer Chemother Pharmacol 1998;42(3):235-40.
PURPOSE: MMDX [3'-deamino-3'-[2(S)-methoxy-4-morpholinyl] doxorubicin], an anthracycline derivative active in vitro and in vivo against multidrug-resistant tumors, is currently under investigation in phase I clinical trials. In vivo it is metabolically activated, resulting in more cytotoxic compounds. We determined in vitro the toxic concentration of a 1-h period of exposure to doxorubicin (DX), MMDX, and bioactivated MMDX on hematopoietic progenitors and tumor cell lines. METHODS: DX and MMDX were tested on both bone marrow- (BM) and cord blood (hCB)-derived clonogenic cells, whereas the metabolites were tested on hCB only. All substances were tested on seven tumor cell lines. RESULTS: BM cells proved to be twice as sensitive as hCB cells to cytotoxics, and MMDX was twice as toxic as DX against hCB cells; MMDX activated with normal rat-liver microsomes and with dexamethasone-induced rat microsomes were, respectively, 70 and 230 times more toxic than MMDX. A comparison of the cytotoxic concentrations on hematopoietic progenitors and tumor cells, revealed that DX and MMDX had 5-fold stronger activity on tumor cell lines than on granulocyte/macrophage colony-forming cells (GM-CFCs), whereas bioactivated MMDX showed comparable cytotoxicity against tumor cells and hematopoietic progenitors. CONCLUSIONS: MMDX metabolites are very potent but display a lower degree of tumor selectivity than MMDX. Strategies to reduce MMDX metabolization should be developed to optimize the therapeutic index of this new anthracycline.

Gilbert ES, Griffith WC, Boecker BB, Dagle GE, Guilmette RA, Hahn FF, Muggenburg BA, Park JF, Watson CR. **Statistical modeling of carcinogenic risks in dogs that inhaled 238PuO2.** Radiat Res 1998;150(1):66-82.
Combined analyses of data on 260 life-span beagle dogs that inhaled 238PuO2 at the Inhalation Toxicology Research Institute (ITRI) and at Pacific Northwest National Laboratory (PNNL) were conducted. The hazard functions (age-specific risks) for incidence of lung, bone and liver tumors were modeled as a function of cumulative radiation dose, and estimates of lifetime risks based on the combined data were developed. For lung tumors, linear-quadratic functions provided an adequate fit to the data from both laboratories, and linear functions provided an adequate fit when analyses were restricted to doses less than 20 Gy. The estimated risk coefficients for these functions were significantly larger when based on ITRI data compared to PNNL data, and dosimetry biases are a possible explanation for this difference. There was also evidence that the bone tumor response functions differed for the two laboratories, although these differences occurred primarily at high doses. These functions were clearly nonlinear (even when restricted to average skeletal doses less than 1 Gy), and evidence of radiation-induced bone tumors was found for doses less than 0.5 Gy in both laboratories. Liver tumor risks were similar for the two laboratories, and linear functions provided an adequate fit to these data. Lifetime risk estimates for lung and bone tumors derived from these data had wide confidence intervals, but were consistent with estimates currently used in radiation protection. The dog-based lifetime liver tumor risk estimate was an order of magnitude larger than that used in radiation protection, but the latter also carries large uncertainties. The application of common statistical methodology to data from two studies has allowed the identification of differences in these studies and has provided a basis for common risk estimates based on both data sets.

Gold LS, Slone TH, Ames BN. **What do animal cancer tests tell us about human cancer risk?: overview of analyses of the carcinogenic potency database.** Drug Metab Rev 1998;30(2):359-404.
Many important issues in carcinogenesis can be addressed using our Carcinogenic Potency Database, which analyzes and standardizes the literature of chronic carcinogenicity tests in laboratory animals. This review is an update and overview of our analyses during the past 15 years, using the current database that includes results of 5152 experiments on 1298 chemicals. We address the following: 1. More than half the 1298 chemicals tested in long-term experiments have been evaluated as carcinogens. We describe this positivity rate for several subsets of the data (including naturally occurring and synthetic chemicals), and we hypothesize and important role in the interpretation of results for increased cell division due to administration of high doses. 2. Methodological issues in the interpretation of animal cancer tests: constraints on the estimation of carcinogenic potency and validity problems associated with using the limited data from bioassays to estimate human risk, reproducibility of results in carcinogenesis bioassays, comparison of lifetable and summary methods of analysis, and summarizing carcinogenic potency when multiple experiments on a chemical are positive. 3. Positivity is compared in bioassays for two closely related species, rats and mice, tested under similar experimental conditions. We assess what information such a comparison can provide about interspecies extrapolation. 4. Rodent carcinogens induce tumors in 35 different target organs. We describe the frequency of chemicals that induce tumors in rats or mice at each target site, and we compare target sites of mutagenic and nonmutagenic rodent carcinogens. 5. A broad perspective on evaluation of possible cancer hazards from rodent carcinogens is given, by ranking 74 human exposures (natural and synthetic) on the HERP indes.

Hagmar L, Bonassi S, Stromberg U, Brogger A, Knudsen LE, Norppa H, Reuterwall C. **Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH).** Cancer Res 1998;58(18):4117-21.
Chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronuclei (MN) in peripheral blood lymphocytes have for decades been used as cytogenetic biomarkers to survey genotoxic risks in the work environment. The conceptual basis for this application has been the idea that increased cytogenetic damage reflects an enhanced cancer risk. Nordic and Italian cohorts have been established to evaluate this hypothesis, and analyses presented previously have shown a positive trend between CA frequency and increased cancer risk. We now report on a pooled analysis of updated data for 3541 subjects examined for CAs, 2703 for SCEs, and 1496 for MN. To standardize for interlaboratory variation, the results for the various cytogenetic end points were trichotomized on the basis of the absolute value distribution within each laboratory as "low" (1-33 percentile),

"medium" (34-66 percentile), or "high" (67-100 percentile). In the Nordic cohort, there was an elevated standardized incidence ratio (SMR) for all cancer among subjects with high CA frequency [1.53; 95% confidence interval (CI), 1.13-2.05] but not for those with medium or low CA frequency. In the Italian cohort, a SMR in cancer of 2.01 (95% CI, 1.35-2.89) was obtained for those with a high CA frequency level, whereas the SMRs for those with medium or low did not noticeably differ from unity. Cox's proportional hazards models gave no evidence that the effect of CAs on total cancer incidence/mortality was modified by gender, age at test, or time since test. No association was seen between the SCEs or the MN frequencies and subsequent cancer incidence/mortality. The present study further supports our previous observation on the cancer predictivity of the CA biomarker, which seems to be independent of age at test, gender, and time since test. The risk patterns were similar within each national cohort. This result suggests that the frequency of CAs in peripheral blood lymphocytes is a relevant biomarker for cancer risk in humans, reflecting either early biological effects of genotoxic carcinogens or individual cancer susceptibility.

Hagmar L, Bonassi S, Stromberg U, Mikoczy Z, Lando C, Hansteen IL, Montagud AH, Knudsen L, Norppa H, Reuterwall C, et al. **Cancer predictive value of cytogenetic markers used in occupational health surveillance programs: a report from an ongoing study by the European Study Group on Cytogenetic Biomarkers and Health.** Mutat Res 1998;405(2):171-8.
The cytogenetic endpoints in peripheral blood lymphocytes: chromosomal aberrations (CA), sister chromatid exchange (SCE) and micronuclei (MN) are established biomarkers of exposure for mutagens or carcinogens in the work environment. However, it is not clear whether these biomarkers also may serve as biomarkers for genotoxic effects which will result in an enhanced cancer risk. In order to assess this problem, Nordic and Italian cohorts were established, and preliminary results from these two studies indicated a predictive value of CA frequency for cancer risk, whereas no such associations were observed for SCE or MN. A collaborative study between the Nordic and Italian research groups, will enable a more thorough evaluation of the cancer predictivity of the cytogenetic endpoints. We here report on the establishment of a joint data base comprising 5271 subjects, examined 1965-1988 for at least one cytogenetic biomarker. Totally, 3540 subjects had been examined for CA, 2702 for SCE and 1496 for MN. These cohorts have been followed-up with respect to subsequent cancer mortality or cancer incidence, and the expected values have been calculated from rates derived from the general populations in each country. Stratified cohort analyses will be performed with respect to the levels of the cytogenetic biomarkers. The importance of potential effect modifiers such as gender, age at test, and time since test, will be evaluated using Poisson regression models. The remaining two potential effect modifiers, occupational exposures and smoking, will be assessed in a case-referent study within the study base. Copyright 1998 Elsevier Science B.V.

Hahm KB, Lee KJ, Kim JH, Cho SW, Chung MH. **Helicobacter pylori infection, oxidative DNA damage, gastric carcinogenesis, and reversibility by rebamipide.** Dig Dis Sci 1998;43(9 Suppl):72s-77s.
Several epidemiological studies have demonstrated a close association between Helicobacter pylori infection and carcinoma of the mid- or distal stomach. If this can be shown to be a causal association, eradication of the organism may prevent later development of cancer. Several mechanisms have been proposed by which H. pylori infection might lead to predisposition for gastric cancer. Although many potential pathogenic mechanisms, such as increased proliferative gastric epithelial response to H. pylori, lowered gastric ascorbic acid levels, and high occurrences of atrophic gastritis, have been proposed, there is little evidence as to which might be of direct importance to such H. pylori-related disease in vivo. H. pylori-associated inflammation may interact with other causal factors related to gastric carcinogenesis and can result in the intestinal type of gastric cancer and then DNA damage due to oxygen radicals induced by persistent inflammatory cell infiltrations in the gastric mucosa may lead to alterations of the gene and result in the development of diffuse-type carcinoma. In order to know the influence of H. pylori on changes of inflammation-related DNA damage, we measured the sequential changes of 8-hydroxydeoxyguanosine (8-OHdG) contents of DNA and the changes of two biomarkers inducible nitric oxide synthase (iNOS) and apoptosis from human gastric mucosa according to the status of H. pylori. The increased levels of oxidative DNA damage, increased occurrences of apoptosis, and increased expressions of iNOS seem to provide the mechanistic links between H. pylori infection and gastric carcinogenesis and rebamipide can abrogate the levels of these hazard factors.

Hamilton JW, Kaltreider RC, Bajenova OV, Ihnat MA, McCaffrey J, Turpie BW, Rowell EE, Oh J, Nemeth MJ, Pesce CA, et al. **Molecular basis for effects of carcinogenic heavy metals on inducible gene expression.** Environ Health Perspect 1998;106(Suppl 4):1005-15.
Certain forms of the heavy metals arsenic and chromium are considered human carcinogens, although they are believed to act through very different mechanisms. Chromium(VI) is believed to act as a classic and mutagenic agent, and DNA/chromatin appears to be the principal target for its effects. In contrast, arsenic(III) is considered nongenotoxic, but is able to target specific cellular proteins, principally through sulfhydryl interactions. We had previously shown that various genotoxic chemical carcinogens, including chromium (VI), preferentially altered expression of several inducible genes but had little or no effect on constitutive gene expression. We were therefore interested in whether these carcinogenic heavy metals might target specific but distinct sites within cells, leading to alterations in gene expression that might contribute to the carcinogenic process. Arsenic(III) and chromium(VI) each significantly altered both basal and hormone-inducible expression of a model inducible gene, phosphoenolpyruvate carboxykinase (PEPCK), at nonovertly toxic doses in the chick embryo in vivo and rat hepatoma H411E cells in culture. We have recently developed two parallel cell culture approaches for examining the molecular basis for these effects. First, we are examining the effects of heavy metals on expression and activation of specific transcription factors known to be involved in regulation of susceptible inducible genes, and have recently observed significant but different effects of arsenic(III) and chromium(VI) on nuclear transcription factor binding. Second, we have developed cell lines with stably integrated PEPCK promoter-luciferase reporter gene constructs to examine effects of heavy metals on promoter function, and have also recently seen profound effects induced by both chromium(VI) and arsenic(III) in this system. These model systems should enable us to be able to identify the critical cis (DNA) and trans (protein) cellular targets of heavy metal exposure leading to alterations in expression of specific susceptible genes. It is anticipated that such information will provide valuable insight into the mechanistic basis for these effects as well as provide sensitive molecular biomarkers for evaluating human exposure.

Hanada T, Isobe H, Saito T, Ogura S, Takekawa H, Yamazaki K, Tokuchi Y, Kawakami Y. **Intracellular accumulation of thallium as a marker of cisplatin cytotoxicity in nonsmall cell lung carcinoma. An application of inductively coupled plasma mass spectrometry.** Cancer 1998;83(5):930-5.
BIOSIS COPYRIGHT: BIOL ABS. BACKGROUND. Thallium-201 (201Tl) scintigraphy has been used to detect malignant pulmonary disease. The mechanism of Tl influx in tumor cells is believed to be similar to that of cisplatin (CDDP) mediated by sodium- and potassium-activated adenosine triphosphatase (Na-K ATPase), and the Na-K ATPase activity may determine the cellular CDDP accumulation and sensitivity to CDDP. The objective of this study was to determine the accumulation of CDDP and Tl in vitro by using inductively coupled plasma mass spectrometry (ICP-MS), a new analytic technique for detecting ultra trace elements, and to evaluate the correlations between cellular CDDP and Tl accumulation, between CDDP 50% inhibitory concentration (IC50) values and cellular CDDP accumulation, and between CDDP IC50 values and cellular Tl accumulation. METHODS. Eight nonsmall cell lung carcinoma (NSCLC) cell lines were used (five adenocarcinomas and three squamous cell carcinomas). The cell lines were exposed to CDDP or Tl for 1 hour, and the resulting cellular accumulation of platinum and Tl was determined by ICP-MS. CDDP IC50 values were determined by a soluble tetrazolium/formazan assay. RESULTS. The authors were able to measure cellular CDDP and Tl accumulation precisely, and heterogeneity in the cellular accumulation of CDDP and Tl existed among the NSCLC cell lines. A significant inverse correlation was observed between CDDP IC50 values and the cellular accumulation of both CDDP and Tl. CONCLUSIONS. ICP-MS is suitable for the determination of cellular CDDP and Tl accumulation in NSCLC cell lines. Cellular Tl accumulation determined by ICP-MS may reflect CDDP cytotoxicity rather than cellular CDDP accumulation.

Hawkins WE, Walker WW, James MO, Manning CS, Barnes DH, Heard CS, Overstreet RM. **Carcinogenic effects of 1,2-dibromoethane (ethylene dibromide; EDB) in Japanese medaka (Oryzias latipes).** Mutat Res 1998;399 (2):221-32.
The carcinogenicity of 1,2-dibromoethane (ethylene dibromide; EDB) was investigated in the Japanese medaka (Oryzias latipes), a small fish species. EDB was administered in water continuously for 97 days to a low concentration group, for 73 days to an intermediate concentration group, and intermittently for 24 h once each week over 97 days to a high concentration group. Medaka were 7 days old at the beginning of the tests. Mean measured

EDB concentrations in the ambient water were 0.13 mg l-1, 6.20 mg l-1, and 18.58 mg l-1 in the low, intermediate, and high concentration groups, respectively. Two control groups, one inside and one outside the exposure apparatus, were used. Samples were examined histologically at 24, 36, and 58 weeks from the beginning of the tests. EDB was clearly carcinogenic to medaka in the intermediate and high concentration groups causing (1) hepatocellular adenomas and carcinomas, (2) cholangiomas, (3) chloangiocarcinomas, and (4) gall bladder papillary adenomas and adenocarcinomas. In separate studies, medaka exposed to 1.0 mg l-1 EDB for 2 to 5 weeks had elevated hepatic glutathione S-transferase activities, possibly indicating induction of a pathway that forms the reactive metabolite of EDB in mammals. SDS-PAGE of hepatic cytosolic fractions of EDB-exposed medaka showed a pronounced increase in a band at 26,000 Da, the expected position for GSH-S-transferase. Although little is known about EDB's mechanisms of action, medaka appear exceptionally sensitive to the carcinogenic effects of EDB and could serve as a model test species for studying similar compounds.

Inoue S, Hasegawa K, Wakamatsu K, Ito S. **Comparison of antimelanoma effects of 4-S-cysteaminylphenol and its homologues.** Melanoma Res 1998;8(2):105-12.
4-S-Cysteaminylphenol (4-S-CAP), a phenolic thioether, has been evaluated for melanocytotoxicity. We have recently shown that dihydro-1,4-benzothiazine-6,7-dione (benzothiazine BQ) is the ultimate toxic metabolite produced by tyrosinase oxidation of 4-SCAP. In this study we compared the antimelanoma effects of 4-SCAP and its two homologues, alpha-methyl-4-S-cysteaminylphenol (alpha-Me-4-SCAP) and 4-S-homocysteaminylphenol (4-S-Homo-CAP). Biochemical experiments showed that upon tyrosinase oxidation alpha-Me-S-CAP and 4-S-Homo-CAP also produced homologues of BQ which reacted rapidly with reduced glutathione (GSH) and also inhibited alcohol dehydrogenase, an SH enzyme. In vitro experiments showed that 4-S-CAP and its two homologues were taken up into B16-F1 melanoma cells at comparable rates but that 4-S-Homo-CAP was least effective in GSH deprivation, which was reflected in the low cytotoxicity of this phenol, and that the cytotoxicity of the phenols was tyrosinase dependent, as proved by the negligible effects on B16-G4F cells which have a much lower tyrosinase activity. In vivo experiments showed that direct intratumoral administration of these phenols inhibited the subcutaneous growth of B16 melanoma, with 4-S-Homo-CAP being the least effective, and that indirect Intraperitoneal administration of 4-S-CAP inhibited melanoma growth much more effectively than the two homologues. These results indicate that 4-S-CAP is the most promising antimelanoma agent among the three phenols examined.

Ishizuka H, Watanabe M, Kubota T, Matsuzaki SW, Otani Y, Kitajima M. **Antitumor activity of murine monoclonal antibody NCC-ST-421 on human cancer cells by inducing apoptosis.** Anticancer Res 1998;18 (4a):2513-8.
BACKGROUND: The murine monoclonal antibody NCC-ST-421 (ST-421) recognizes dimeric Le(a) antigen expressed on gastrointestinal cancer cells. MATERIALS AND METHODS: Direct antitumour activity of ST-421 was evaluated using dimeric Le(a) positive cell lines Colo 205, Colo 201, HT-29 and WiDr; and negative cell lines MX-1 and K562. RESULTS: While time- and concentration-dependent antitumour activity was observed against Colo 205 and Colo 201, no antitumour activity was detected against the other cell lines tested in an in vitro cytotoxicity assay. When ST-421 was administered intraperitoneally daily for 2 weeks to severe combined immunodeficient (SCID) mice transplanted with tumour xenografts, inhibition of tumour growth was observed against Colo 205, and to a lesser extent HT-29 and WiDr. Anti-asialo GM1 antibody did not block this antitumour activity, suggesting ST-421 has a direct cytotoxic effect. The degree of antitumour activity of ST-421 dependent on the grade of Le(a)-expression as detected by immunohistochemical staining. CONCLUSIONS: Flow cytometric and immunohistochemical analysis suggests the induction of apoptosis may play a key role in the direct antitumour activity of ST-421 on Colo 205 cells.

Jankowiak R, Ariese F, Hewer A, Luch A, Zamzow D, Hughes NC, Phillips D, Seidel A, Platt KL, Oesch F, et al. **Structure, conformations, and repair of DNA adducts from dibenzo(a,l)pyrene: 32P-postlabeling and fluorescence studies.** Chem Res Toxicol 1998;11(6):674-85.
BIOSIS COPYRIGHT: BIOL ABS. The nature of stable DNA adducts derived from the very potent carcinogen dibenzo(a,l)pyrene (DB(a,l)P) in the presence of rat liver microsomes in vitro and in mouse skin in vivo has been

studied using 32P-postlabeling and laser-based fluorescence techniques. Analysis of DB(a,l)P-DNA adducts via 32P-postlabeling has been obtained by comparison of the adduct patterns to those obtained from reactions of synthetic (:)-anti-, (+)-anti-, (-)-anti-, and (:)-syn-DB(a,l)P11,12-diol 13,14-epoxide (DB(a,l)PDE) with single nucleotides and calf thymus DNA. antiDB(a,l)PDE-dA adducts derived from the (-)-enantiomer are the major adducts formed in calf thymus DNA and in mouse skin DNA. The ratio of deoxyadenosine to deoxyguanosine modification is approximately 2:1 in mouse skin exposed to DB(a,l)P; activation by rat liver microsomes leads to a similar profile of adducts but with two additional spots. The conformations of DB(a,l)P adducts in native DNA, as well as the possibility of conformation dependent repair, have been explored by low-temperature fluorescence spectroscopy. These studies have been performed using polynucleotides and calf thymus DNA reacted in vitro with DB(a,l)PDE and native DNA from mouse epidermis exposed to DB(a,l)P. The results show that adducts are heterogeneous, possess different structures, and adopt different conformations. External, external but base-stacked and intercalated adduct conformations are observed in calf thymus DNA and in mouse skin DNA samples. Differences in adduct repair rates are also revealed; namely, the analysis of mouse skin DNA samples obtained at 24 and 48 h after exposure to DB(a,l)P clearly shows that external adducts are repaired more efficiently than intercalated adducts. These results, taken together with those for B(a)P-DNA adducts (Suh et al. (1995) Carcinogenesis 16, 2561-2569), indicate that the repair of DNA damage resulting from PAH diol epoxides is conformation-dependent.

Jones TD, Morris MD, Basavaraju SR. **Atherosclerotic risks from chemicals: Part II. A RASH analysis of in vitro and in vivo bioassay data to evaluate 45 potentially hazardous compounds.** Arch Environ Contam Toxicol 1998;35(1):165-77.
As reviewed in the Part I companion manuscript by Basavaraju and Jones (Arch Environ Contam Toxicol), atherosclerosis and carcinogenesis may share some common mechanisms of toxicological action. On that hypothesis, standardized test data taken from the Registry of Toxic Effects of Chemical Substances (RTECS) were used to compute relative potency factors for chemical compounds associated with increased risk of atherosclerosis to humans. Potencies of the different compounds were computed relative to each of six reference compounds comprised of benzo(a)pyrene, nicotine, cisplatin, adriamycin, estrogen, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Reference-specific potencies were all converted to a common numerical scale adjusted to unit potency for B(a)P. Because the list of compounds contained several antibiotics, amino acids, hormones, chemotherapeutic agents, polynuclear aromatics, alkaloids, metals, and vitamins, the standardized estimates of potency varied significantly depending on which of the six reference compounds are considered as standards of comparison. For the n - 1 other substances. Estimates of relative potency, risk coefficients, and generalized risk equations are estimated for cigarette smoke condensate, dietary cholesterol, ethanol, and carbon disulfide. From data on atherosclerosis as a result of cigarette smoking, a tentative risk was estimated as Increased Relative Risk = S (mg/kg-day)-1 x dose (mg/kg-day) x RP, where the dose is chronic intake per kilogram of body weight per day, RP is the potency of the compound of interest relative to that of benzo(a)pyrene, and S is 0.83, 0.25, 0.20, or 13 depending on whether cigarette smoke, cholesterol, ethanol, or carbon disulfide epidemiological data were used as a standard of comparison.

Kanitz MH, Li EI, Schulte PA, Anderson NL, Rothman N, Savage RE Jr. **Investigation of ornithine decarboxylase activity and two-dimensional electrophoretic protein profile following exposure of T24 bladder carcinoma cells to tumor promoter and carcinogen.** Toxicol Methods 1997;7(1):27-41.
Changes were investigated in T24 human bladder carcinoma cell ornithine-decarboxylase activity and protein profiles in an effort to develop appropriate screening tools for biomarkers of effects of exposure to occupational chemicals. A unique chromatographic approach was used to demonstrate that in-vitro exposure of T24 cells for 6 hours to varying concentrations of 4-aminobiphenyl (92671) elevated enzyme activity 5.3 to 5.9 fold. Two dimensional gel electrophoresis was also used to compare the protein pattern of vehicle control treated T24 to 4-aminobiphenyl or 12-o-tetradecanoylphorbol-13-acetate treated cells. The Kepler software package was used to determine changes in abundance and modification of proteins. Protein markers were identified by significant changes in spot density following T24 treatment with either chemical. Fifteen protein spots from a detectable pool of 542 demonstrated two fold or greater changes in intensity. These findings illustrate the potential of automated two

dimensional gel analysis for classifying different gel patterns. In addition to the ornithine-decarboxylase assay, two dimensional polyacrylamide gel electrophoresis offers promise for evaluating potential biomarkers for occupational and environmental carcinogens. The authors suggest that their findings will assist NIOSH in their efforts in the molecular epidemiology of occupational bladder carcinogenesis.

Kapadia GJ, Tokuda H, Sridhar R, Balasubramanian V, Takayasu J, Bu P, Enjo F, Takasaki M, Konoshima T, Nishino H. **Cancer chemopreventive activity of synthetic colorants used in foods, pharmaceuticals and cosmetic preparations.** Cancer Lett 1998;129(1):87-95.
In continuation with our studies to uncover cancer chemopreventive effects of non-toxic natural colorants and other products of biologic and synthetic origin, we tested several Food and Drug Administration-approved synthetic colorants for antitumor promoting potential by the in vitro Epstein-Barr virus early antigen activation in Raji cells in response to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Among 29 such colorants used in foods, pharmaceuticals and cosmetics and evaluated in vitro, six of the 10 most effective had an azo group. Three structurally unrelated colorants tested in this assay were also studied in vivo for chemoprevention of 7,12-dimethylbenz[a]anthracene (DMBA)-induced TPA-promoted mouse skin carcinogenesis. The results indicate that tartrazine, indigo carmine and erythrosine are potent inhibitors of skin tumor promotion in mice treated with DMBA and TPA.

Kato Y, Flodstrom S, Warngard L. **Initiation and promotion of altered hepatic foci in female rats and inhibition of cell-cell communication by the imidazole fungicide prochloraz.** Chemosphere 1998;37(3):393-403.
BIOSIS COPYRIGHT: BIOL ABS. The imidazole fungicide prochloraz (1-(-propyl-N-2,4,6-trichlorophenoxy)ethyl carbamoyl)imidazole) was investigated for its ability to inhibit gap junctional intercellular communication in the scrape-loading/dye-transfer assay in IAR 20 rat liver epithelial cells and for effects on the initiation and promotion stages of hepatocarcinogenesis. Female Sprague-Dawley rats initiated with N-nitrosodimethylamine 24-hr after partial hepatectomy were administered prochloraz five days a week by oral gavage (30 or 150 mg/kg) for 10 weeks. Altered hepatic foci (AHF) were analyzed by quantitative stereology from liver sections stained for gamma-glutamyltranspeptidase (GGT) and glutathione S-transferase P (GST-P). The fungicide was also studied for its ability to initiate the development of GGT-positive AHF in rat liver. The in vitro studies showed prochloraz to be an inhibitor of cell-cell communication in test system used. In vivo studies, prochloraz showed no effect on the initiation of GGT-positive foci. However, significant increases in the percentage of liver tissue occupied by GGT-positive AHF and the number of GST-P-positive AHF per cm3 in initiated animals were recorded in the low dose group. The present data suggest that prochloraz acts as a weak tumour promoter of hepatocarcinogenesis but does not initiate this process.

Kawabata K, Nio Y, Imamura M, Fukumoto M. **Anticancer chemosensitivity and growth rate of freshly separated human colorectal cancer cells assessed by in vitro DNA synthesis inhibition assay.** Anticancer Res 1998;18(3a):1633-40.
The present study was designed to assess the chemosensitivity profile of freshly separated colorectal cancer cells and to screen effective agents for the design of new combination regimens. The DNA synthesis and chemosensitivity (% inhibition of DNA synthesis by the anticancer agent) were successfully assessed in 184 samples (107 primary and 77 metastatic or recurrent lesions) from 152 patients with colorectal cancer using an 3H-thymidine incorporation assay, and the correlations between these two measures and various clinicopathological factors were analysed. DNA synthesis was highest in nodal metastasis followed by malignant effusion, primary lesion, liver metastasis, and local recurrence. DNA synthesis liver metastasis and local recurrence were significantly lower than in other lesions. The results of the chemosensitivity assay are as follows: 5-FU seems to be the most beneficial for primary colorectal cancer; carboquone (CQ), etoposide (VP-16), 5-FU, and mitomycin-C (MMC) for nodal metastasis; CQ, cisplatin (CDDP), 5-FU, adriamycin (ADR) and VP-16 for malignant effusion; and VP-16, CDDP and CQ for liver metastasis. However, the present results showing the chemosensitivity profiles in different lesions suggest that regimens including 5-FU with VP-16 and CQ in addition to MMC or ADR may be applicable for all kinds of colorectal cancer lesions. These results demonstrated the heterogeneity in the chemosensitivity of colorectal cancer, which suggests not only the necessity of patient-specific chemotherapy dependent on the

sensitivity assay, but also the usefulness of the present results in the choice of agents for widely applicable combination regimens for colorectal cancer.

Keenan KP, Laroque P, Dixit R. **Need for dietary control by caloric restriction in rodent toxicology and carcinogenicity studies.** J Toxicol Environ Health B Crit Rev 1998;1(2):135-48.
The conditions under which laboratory animals are maintained can powerfully influence the results of toxicological studies utilized for risk assessment. Nutrition is of importance in toxicological bioassays and research, because diet composition and the conditions under which it is fed can affect the metabolism and activity of xenobiotic test substances and alter the results and reproducibility of long-term studies. It is known that ad libitum (AL) overfed sedentary laboratory rodents suffer from an early onset of degenerative disease and diet-related tumors that lead to poor survival in chronic bioassays. AL-fed animals are not well-controlled subjects for any experimental studies. Examination of study-to-study variability in food consumption, body weight, and survival in carcinogenicity studies for the same strain or stock of rodents shows tremendous laboratory-to-laboratory variability. However, a significant correlation between average food (calorie) consumption, adult body weight, and survival has been clearly established. The use of moderate dietary restriction (DR) results in a better controlled rodent model with a lower incidence or delayed onset of spontaneous diseases and tumors. Operationally simple, moderate DR significantly improves survival, controls adult body weight and obesity, reduces age-related renal, endocrine, and cardiac diseases, increases exposure time, and increases the statistical sensitivity of these expensive, chronic bioassays to detect a true treatment effect. A moderate DR regimen of 70-75% of the maximum unrestricted AL food intake is recommended as a nutritionally intelligent, well-established method in conducting well-controlled toxicology and carcinogenicity studies.

Kensler TW, Groopman JD, Roebuck BD. **Use of aflatoxin adducts as intermediate endpoints to assess the efficacy of chemopreventive interventions in animals and man.** Mutat Res 1998;402(1-2):165-72.
Clinical cancer prevention studies that use disease as an endpoint are of necessity, large, lengthy, and extremely costly. Development of the field of cancer chemoprevention is being accelerated by the application of intermediate markers to preclinical and clinical studies. Sensitive and specific analytic methods have been developed for detecting and quantifying levels of covalent adducts of aflatoxins with cellular DNA and blood proteins at ambient levels of exposure. Such biomarkers can be applied to the preselection of exposed individuals for study cohorts, thereby reducing study size requirements. Levels of these aflatoxin-DNA and albumin adducts can be modulated by chemopreventive agents such as oltipraz and chlorophyllin in experimental models. Overall, a good concordance is seen between diminution of biomarkers and reductions in tumor incidence and/or multiplicity in these settings. Thus, these markers can also be used to rapidly assess the efficacy of preventive interventions. However, the successful application of these biomarkers to clinical prevention trials will be dependent upon prior determination of the associative or causal role of the marker to the carcinogenic process, establishment of the relationship between dose and response, and appreciation of the kinetics of adduct formation and removal. The general approach that has been utilized for the development, validation and application of aflatoxin-DNA and protein adduct biomarkers to cancer chemoprevention trials is summarized.

Kerckaert GA, Leboeuf RA, Isfort RJ. **Assessing the predictiveness of the Syrian Hamster Embryo Cell Transformation Assay for determining the rodent carcinogenic potential of single ring aromatic/ nitroaromatic amine compounds.** Toxicol Sci 1998;41(2):189-97.
 The pH 6.7 Syrian hamster embryo (SHE) cell transformation assay was used to test the morphological transformation potential of 5 rodent carcinogenic single ring aromatic/nitroaromatic amine compounds: 2-amino-4-nitrotoluene, 2,4-diaminotoluene, 2,4-dinitrotoluene, o-anisidine hydrochloride, and o-toluidine; and 5 noncarcinogenic single ring aromatic/nitroaromatic amine compounds: 2,6-diaminotoluene, 2,4-dimethoxyaniline hydrochloride, 4-nitro-o-phenylenediamine, p-phenylenediamine dihydrochloride, and HC Blue No. 2. All 5 rodent carcinogens produced significant morphological transformation in a dose-responsive manner. None of the 5 noncarcinogens yielded significant transformation at any of the doses tested. Therefore, the concordance between the pH 6.7 SHE cell transformation assay and rodent carcinogenicity for these 10 single ring aromatic/ nitroaromatic amine compounds is 100%. In contrast, the concordance between the standard SHE cell

transformation assay and rodent carcinogenicity for 13 single ring aromatic/nitroaromatic amine compounds was 62%. For 5 aromatic/nitroaromatic amine compounds which were tested in both standard and pH 6.7 SHE cell transformation assays (i.e., a subset of the above two databases), the concordance between the standard SHE cell transformation assay and the rodent bioassay was 40%, while the concordance between the pH 6.7 SHE cell transformation assay and the rodent bioassay was 100%. This relatively high concordance between the pH 6.7 SHE cell transformation assay and rodent bioassay results demonstrates the utility of the pH 6.7 SHE cell transformation assay for predicting the rodent carcinogenic potential of single ring aromatic/nitroaromatic amine compounds.

Konstantinov SM, Topashka-Ancheva M, Benner A, Berger MR. **Alkylphosphocholines: effects on human leukemic cell lines and normal bone marrow cells.** Int J Cancer 1998;77(5):778-86.
The anti-leukemic activity of a series of alkylphosphocholines (APCs) was studied against a panel of human leukemic cell lines (HL-60, K-562, Reh, MOLT-4, Jurkat, Ramos and Raji). Cytotoxic efficacy was measured by the MTT cell survival assay. All cell lines were found to be sensitive, except the multipotential CML-derived K-562 cell line. Flow cytometry of HL-60 cells showed a significant decrease of cells in S phase and the formation of a sub-G fraction. DNA fragmentation typical for programmed cell death was detected by DNA gel electrophoresis in these cells but not in any of the other leukemic lines. At concentrations below the cytotoxic range, mitogenic effects were seen in HL-60 cells after 14-hr exposure. Colony formation by K-562 cells revealed an augmented clonogenicity after exposure to APC with a short alkyl chain. In contrast, cells of lymphoid origin did not undergo DNA fragmentation or show mitogenic stimulation after exposure to APC. Normal bone marrow cells were also investigated for mitogenic and genotoxic effects. No decrease was found in the number of hematopoietic progenitors in long-term bone marrow cell cultures after exposure to APC. On the contrary, a significant increase was found after short exposure. Dodecylphosphocholine, hexadecylphosphocholine (HPC) and (octadecyl-[2-(N-methylpiperidino)-ethyl]phosphate exhibited a mild clastogenicity at equimolar high doses on murine bone marrow cells in vivo, which is unusual for the majority of classical DNA-interacting anti-cancer drugs. In conclusion, APCs are agents with a broad spectrum of in vitro anti-leukemic effects, which lack hematological toxicity.

Lash LH, Qian W, Putt DA, Desai K, Elfarra AA, Sicuri AR, Parker JC. **Glutathione conjugation of perchloroethylene in rats and mice in vitro: sex-, species-, and tissue-dependent differences.** Toxicol Appl Pharmacol 1998;150(1):49-57.
BIOSIS COPYRIGHT: BIOL ABS. Perchloroethylene (Per)-induced nephrotoxicity and nephrocarcinogenicity have been associated with metabolism by the glutathione (GSH) conjugation pathway to form S-(1,2,2-trichlorovinyl) glutathione (TCVG). Formation of TCVG was determined in incubations of Per and GSH with isolated renal cortical cells and hepatocytes from male and female Fischer 344 rats and with renal and hepatic cytosol and microsomes from male and female Fischer 344 rats and B6C3F1 mice. The goal was to assess the role of metabolism in the sex and species dependence of susceptibility to Per-induced toxicity. A key finding was that GSH conjugation of Per occurs in kidney as well as in liver. Although amounts of TCVG formation in isolated kidney cells and hepatocytes from male and female rats were generally similar, TCVG formation in subcellular fractions showed marked sex, species, and tissue dependence. This may be due to the presence of multiple pathways for metabolism in intact cells, whereas only the GSH conjugation pathway is active in the subcellular fractions under the present assay conditions. TCVG formation in kidney and liver subcellular fractions from both male rats and mice were invariably higher than corresponding values in female rats and mice. Amounts of TCVG formation in rat liver subcellular fractions were approximately 10-fold higher than in corresponding fractions from rat kidney. Although rats are more susceptible to Per-induced renal tumors than mice, amounts of TCVG formation were 7- to 10-fold higher in mouse kidney subcellular fractions and 2- to 5-fold higher in mouse liver subcellular fractions of both sexes compared to corresponding fractions from the rat. Hence, although the higher amounts of TCVG formation in liver and kidney from male rats correspond to their higher susceptibility to Per-induced renal tumors compared with female rats, the markedly higher amounts of TCVG formation in mice compared with rats suggest that other enzymatic or transport steps in the handling of Per in mice contribute to their relatively low susceptibility to Per-induced renal tumors.

Liteplo RG, Long GW, Meek ME. **Relevance of carcinogenicity bioassays in mice in assessing potential health risks associated with exposure to methylene chloride.** Human Exper Toxicol 1998;17(2):84-7.

The relevancy of carcinogenicity bioassays in mice for evaluating the potential health risks of methylene-chloride (75092) was discussed. The evidence for methylene-chloride being carcinogenic was summarized. A 2 year carcinogenicity bioassay in which B6C3F1-mice and Fischer-344/N-rats were exposed to up to 4,000 parts per million methylene-chloride vapor found that methylene-chloride caused dose related increases in the number of male and female mice bearing multiple lung or liver tumors. An increased incidence of benign mammary tumors was also seen in female rats. Based on these results and data relating to its genotoxicity, methylene-chloride was classified as a probable carcinogen by the EPA and the Government of Canada and as a possible carcinogen by the International Agency for Research on Cancer. Studies investigating possible mechanisms for methylene-chloride carcinogenicity and their relevancy to human risk assessment were summarized. Studies have suggested that the mouse is an inappropriate model for human risk assessment because a qualitative difference in the subcellular localization of theta class glutathione-S-transferase (GST/T) exists between murine and human tissues. The results of a critical review of data from studies examining the metabolism of methylene-chloride and the subcellular localization of GST/T were discussed. Studies conducted in a number of intact cells such as Chinese-hamster-ovary cells have not found that the genotoxic metabolite S-chloromethylglutathione has to be generated within the nucleus to react with genomic DNA. Studies utilizing mRNA hybridization techniques have not shown conclusively that GST/T is not found in human cells. The authors conclude that there is insufficient evidence to support the view that qualitative differences in the subcellular distribution of GST/T between mice and humans make the mouse an inappropriate model for evaluating the carcinogenic risk of methylene-chloride to humans.

Lo YL, Hsu CY, Huang JD. **Comparison of effects of surfactants with other MDR reversing agents on intracellular uptake of epirubicin in Caco-2 cell line.** Anticancer Res 1998;18(4c):3005-9.
P-glycoprotein (P-gp) actively pumps out a number of anticancer drugs, such as epirubicin, from tumor cells. P-gp is also expressed in the small intestine under normal physiological conditions. Inhibition of intestinal P-gp function using MDR reversing agents may enhance the oral bioavailability of some chemotherapeutic agents. Human colon adenocarcinoma (Caco-2) cell line expresses many characteristics of differentiated cells of the normal small intestine. Using Caco-2 as an in vitro intestinal model, the overall goal of the present study was to evaluate the MDR-reversing effects of some commonly used nonabsorptive pharmaceutical surfactants, such as Tween 20, Tween 80 and acacia on the intracellular accumulation of epirubicin by flow cytometry. Tween 20, Tween 80 or acacia all significantly increased intracellular accumulation of epirubicin with the highest enhancing effect for acacia and the lowest for Tween 20. Apart from progesterone, the enhancing effects of surfactants were better than those of non-surfactant MDR reversing agents such as verapamil, trifluoperazine and reserpine. In conclusion, our results demonstrate that progesterone, acacia, Tween 20 and Tween 80 are potent MDR modifiers of epirubicin in Caco-2 at concentrations that could be achieved in vivo. Use of surfactants in excipients may increase the intestinal absorption of some drugs through P-gp inhibition and thus improve drug bioavailability for P-gp substrate.

Maerz WJ, Baselga J, Reuter VE, Mellado B, Myers ML, Bosl GJ, Spinella MJ, Dmitrovsky E. **FGF4 dissociates anti-tumorigenic from differentiation signals of retinoic acid in human embryonal carcinomas.** Oncogene 1998;17(6):761-7.
A subset of male germ cell cancers presenting with advanced stage abundantly express the fibroblast growth factor-4 (FGF4). FGF4 expression is restricted in vitro to undifferentiated embryonal carcinomas (ECs). During induced differentiation, FGF4 expression is repressed in maturation sensitive but not resistant human ECs, suggesting FGF4 plays an important role in malignant growth or differentiation of ECs. To explore these FGF4 signals in male germ cell cancers, the multipotent human EC NTERA-2 clone D1 (NT2/D1) cell line was studied. All-trans-retinoic acid (RA)-treatment of these cells induces a neuronal phenotype and represses tumorigenicity and FGF4 expression. In contrast, RA-treatment of retinoid resistant lines derived from NT2/D1 cells failed to repress FGF4 expression. This implicated FGF4 directly in regulating human EC growth or differentiation. To evaluate further this FGF4 role, FGF4 was constitutively over-expressed in NT2/D1 cells using a CMV-driven expression vector containing the neomycin resistance gene. Three stable transfectants expressing exogenous FGF4 were studied as was a control transfectant only expressing the neomycin resistance gene. RA-treatment repressed endogenous but not exogenous FGF4 expression. RA-treatment of these transfectants induced morphologic and immunophenotypic maturation, changes in RA-regulated genes, and a G1 cell cycle arrest in a manner similar to parental NT2/D1 cells. This indicated FGF4 over-expression did not block RA-mediated differentiation. As expected, RA-treatment

repressed tumorigenicity of the control transfectant after subcutaneous injection into athymic mice. Despite RA-treatment, this repressed tumorigenicity was overcome in all the transfectants over-expressing FGF4. The histopathology and neovascularization did not appreciably differ between xenograft tumors derived from FGF4 over-expressing versus control transfectants. FGF4 expression studies were extended to patient-derived germ cell tumors using total cellular RNA Northern analysis and an immunohistochemical assay developed to detect FGF4 protein expression. Germ cell tumors with EC components were significantly more likely to express FGF4 mRNA (P < or = 0.0179) than other examined germ cell tumors without EC components. Immunohistochemical results from 43 germ cell tumors demonstrated increased FGF4 expression especially in non-seminomas having EC components. Thus, FGF4 promotes directly malignant growth of cultured ECs, overcomes the antitumorigenic actions of RA, and is selectively expressed in specific histopathologic subsets of germ cell tumors. Taken together, these findings indicate how differentiation and anti-tumorigenic retinoic acid signals can be dissociated in germ cell cancer.

Martelli A, Campart GB, Canonero R, Carrozzino R, Mattioli F, Robbiano L, Cavanna M. **Evaluation of auramine genotoxicity in primary rat and human hepatocytes and in the intact rat.** Mutat Res 1998;414(1-3):37-47.
Auramine, a dye previously found to be a liver carcinogen in both mice and rats, was evaluated for its DNA-damaging and clastogenic activities in primary cultures of rats and human hepatocytes and for the induction of DNA single-strand breaks in the liver and urinary bladder mucosa of intact rats. A similar dose-dependent frequency of DNA fragmentation was revealed by the alkaline elution technique in metabolically competent primary cultures of both rat and human hepatocytes exposed for 20 h to subtoxic concentrations ranging from 10 to 32 microM. In contrast, neither rat nor human hepatocytes displayed an increased frequency of micronuclei after a 48-h exposure to the same auramine concentrations. In rats given a single oral dose of 125, 250 or 500 mg kg-1 auramine, the Comet assay revealed a significant increase in the frequency of DNA lesions in the liver and in the urinary bladder mucosa, the effect being slightly more marked in the liver. Taken as a whole and compared with previous findings, these results suggest that auramine is biotransformed into reactive species in target organs of both rats and humans, and that this dye might play by itself the main role in the increased incidence of bladder cancer which has been judged as causally related to its manufacture. Copyright 1998 Elsevier Science B.V.

Massart C, Gibassier J, Denais A, Genetet N. **Effect of PSC 833 on the efficacy of doxorubicin in vitro in a medullary thyroid carcinoma cell line.** Anticancer Res 1998;18(4c):2953-6.
In medullary carcinoma of the thyroid (MTC), multidrug resistance (MDR) remains the major obstacle to effective chemotherapy. In this work MDR was investigated in TT cells, a human MTC cell line. We studied the effect of an efficient MDR agent (SDZ PSC 833) on doxorubicin (DOX)-induced cytotoxicity in TT cells cultured in monolayers. The toxicity was evaluated with four tests: MTT test, lacticode-hydrogenase and glutathione assays, and neutral red uptake. PSC 833 (3 microM) partially reversed the resistance to DOX in vitro after a 48-hour coincubation, followed by a 24 hour-post incubation. Under these conditions, PSC 833 was not toxic at the concentration used. Our results suggest that PSC 833 has the potential to reverse the MDR phenotype in MTC cells.

Mill AJ, Frankenberg D, Bettega D, Hieber L, Saran A, Allen LA, Calzolari P, Frankenberg-Schwager M, Lehane MM, Morgan GR, et al. **Transformation of C3H 10T1/2 cells by low doses of ionising radiation: a collaborative study by six European laboratories strongly supporting a linear dose-response relationship [see comments].** J Radiol Prot 1998;18(2):79-100.
For the assessment of radiation risk at low doses, it is presumed that the shape of the low-dose-response curve in humans for cancer induction is linear. Epidemiological data alone are unlikely to ever have the statistical power needed to confirm this assumption. Another approach is to use oncogenic transformation in vitro as a surrogate for carcinogenesis in vivo. In mid-1990, six European laboratories initiated such an approach using C3H 10T1/2 mouse cells. Rigid standardisation procedures were established followed by collaborative measurements of transformation down to absorbed doses of 0.25 Gy of x-radiation resulting in a total of 759 transformed foci. The results clearly support a linear dose-response relationship for cell transformation in vitro with no evidence for a threshold dose or for an enhanced, supralinear response at doses approximately 200-300 mGy. For radiological protection this represents a large dose, and the limitations of this approach are apparent. Only by understanding the fundamental

mechanisms involved in radiation carcinogenesis will further knowledge concerning the effects of low doses become available. These results will, however, help validate new biologically based models of radiation cancer risk thus providing increased confidence in the estimation of cancer risk at low doses.

Monro AM, MacDonald JS. **Evaluation of the carcinogenic potential of pharmaceuticals. Opportunities arising from the International Conference on Harmonisation.** Drug Saf 1998;18(5):309-19.
The evaluation of the carcinogenic potential of pharmaceuticals is currently undergoing dramatic changes. For the past 25 years the regulatory expectation for agents intended for long term use has been that lifespan studies (usually lasting 2 years) in 2 rodent species be conducted. These studies take at least 3 years to plan, execute and interpret, and use over 1200 animals. It is now recognised that the quality of the information obtained from these studies is unreliable for prediction of carcinogenic risk to humans. Over the past 4 years, the International Conference on Harmonisation (ICH) has recommended changes in approaches to assessing the carcinogenic potential of pharmaceuticals. In future, only one long term rodent study will be routinely required (usually in rats), provided this is complemented with a short or medium term test in one of the emerging new models for carcinogenicity, such as transgenic mice or newborn mice. However, the relevance of these new models to human cancer and their use in risk assessment is still largely unknown and this situation must be kept under review as knowledge accumulates. A long term study in a second rodent species is still an option. Dose selection has also been improved inasmuch as there are now several alternatives to the use of the maximum tolerated dose (MTD). In the past, the use of the MTD, when the normal homeostasis of the test animals is disturbed, has been considered one of the major problems with the rodent carcinogenicity bioassay. However, one of the alternative end-points to the use of the MTD, i.e. the comparison of plasma concentrations in rodents and humans, must be viewed with caution. While this may contribute to limiting the high dose level for agents of very low toxicity, the concept should not be interpreted as signifying that plasma concentrations provide a sound basis for comparing the carcinogenic activity of agents in different species. Recognition of the 4 properties (genotoxicity, immunosuppression, steroid hormonal activity and long term tissue damage), at least one of which is associated with each of the pharmaceuticals known to be carcinogenic to humans, should focus more attention on a search for these properties in patients. Absence of these properties at clinically relevant dose levels indicates that a pharmaceutical is highly unlikely to be carcinogenic to humans.

Oki E, Sakaguchi Y, Toh Y, Oda S, Maehara Y, Yamamoto N, Sugimachi K. **Induction of apoptosis in human tumour xenografts after oral administration of uracil and tegafur to nude mice bearing tumours.** Br J Cancer 1998;78(5):625-30.
Various types of anti-neoplastic agents induce apoptosis in vitro, but less is known of the role of this mode of cell death in tumours treated in vivo. We examined the induction of apoptosis by oral anti-neoplastic agents, tegafur and uracil (UFT, a combined preparation of 1 mol tegafur and 4 mol uracil), and the relationship of effects on tumour growth. Seven different human gastrointestinal tumour xenografts were transplanted into nude mice, including two colon adenocarcinomas (KM20C and Col-1), three gastric carcinomas (SC-6, St-40 and 4-1ST) and two pancreatic carcinomas (PAN-4 and PAN-12), followed by oral administration of UFT (24 mg kg(-1) day(-1)) for 9 days. The percentage of apoptotic cells in each tumour was scored in histological sections, chronologically, using a molecular biological-histochemical system and growth inhibition was examined in each tumour. A significant growth inhibition by UFT was observed for all tumours, except PAN-12. In KM20C and SC-6, growth inhibition rates were 61.7% and 60.6% respectively. Quantitative assay for apoptosis showed a remarkable induction of apoptosis in KM20C (4.2%) and SC-6 (3.5%), which were relatively sensitive to UFT. In addition, KM20C and SC-6 showed a higher incidence of spontaneous apoptosis. In five other tumours, which responded to a lesser extent than KM20C and SC-6, UFT altered little the changes in apoptosis (less than 2%) and spontaneous apoptosis was relatively low. Thus, tumours with a higher apoptosis induced by UFT had a higher response to UFT. Apoptosis observed in tumours might serve as a predictor of a preferable response to UFT.

Olivo S, Wargovich MJ. **Inhibition of aberrant crypt foci by chemopreventive agents.** In Vivo 1998;12(2):159-66.
The colon carcinogenic process is believed to begin with both genetic and morphological alterations in a few

individual crypts. These select crypts, called aberrant crypt foci (ACF), are widely agreed upon as precursors of colon cancer. The ACF assay involves testing potential chemopreventive agents by counting the number of ACF in a carcinogen-treated colon. This assay has the advantage of not only being less expensive and time-consuming than tumor-producing studies, but will also allow the elucidation of the colon carcinogenic process by letting the researcher explore the changes that occur at a pre-cancerous stage. The ACF assay has been used most frequently in rodent models. In the rodent colon, ACF are easy to distinguish due to their distinct morphological, histological, and biological characteristics. In addition, the ACF assay has been used to look at specific genetic alterations in the crypts such as K-ras, p53, and APC mutations. Our laboratory has consistently used the ACF assay to test many potential chemopreventive agents using rats induced by the colon carcinogen azoxymethane (AOM). Potential chemopreventive agents have been tested in both the initiation or the post-initiation period Research supports the notion that aberrant crypt foci represent a true neoplastic lesion for colon cancer. By studying these lesions both grossly and genetically it may be possible to learn more about the causes of colon carcinogenesis. In addition, by testing new compounds through the ACF assay, it is possible not only to discover potentially new chemopreventive compounds, but also to discover the mechanisms behind them. Because of this, the ACF assay is an invaluable method that will help reveal the process of colon carcinogenesis.

Savage RE Jr, Debord DG, Swaminathan S, Butler MA, Snawder J, Kanitz MH, Cheever K, Reid T, Werren D. **Occupational applications of a human cancer research model.** J Occup Environ Med 1998;40(2):125-35.
An in-vitro/in-vivo human uroepithelial cell (SV-HUCPC) transformation system used to investigate several molecular events that occur along the continuum of exposure to disease outcome as potential biomarkers for occupational carcinogenesis was described. Fourteen athymic nude mice were inoculated with SV-HUCPC treated in-vitro with one of three selected concentrations of N-hydroxy-4,4'-methylenebis(2-chloroaniline) (N-OH-MOCA). Only two of the high dose treated SV-HUCPC resulted in tumors when inoculated into athymic nude mice. Tumors occurred from a total pool of six athymic nude mice inoculated with the high dose treated SV-HUCPC. These findings, and the failure to replicate positive findings in a later study, suggest limitations in the transformation system. As the positive results were only observed in the responsive SV-HUCPC treated cells and there were no tumors in the negative controls, the implication is that the tumorigenic effect is authentic. The results presented illustrate the complexities associated with using this approach for assessing complete transformation of human cells. The authors conclude that the human uroepithelial cell culture model may be a highly relevant mechanism to study human occupational bladder carcinogenesis and biomarker development by arylamines.

Schwetz B, Gaylor D. **Alternative tests: carcinogenesis as an example.** Environ Health Perspect 1998;106(Suppl 2):467-71.
Acceptance of new tests that are alternatives to currently used toxicology tests is a topic of considerable importance in the field of toxicology. Carcinogenicity testing today normally includes 2-year studies in rats and mice of both sexes, following widely accepted procedures for husbandry; selection of dose levels; pathology and toxicity observations; and statistical interpretation of tumor data. These studies are usually preceded by tests for genetic toxicity and subchronic toxicity studies to select dose levels for the 2-year studies. Although these data are used for quantitative risk assessment, the mechanistic basis for effects is usually unknown. The series of studies is very expensive and requires 5 years or more to conduct. Alternative approaches are being developed that would provide more mechanistic information and hopefully would permit decisions to be made about carcinogenic potential without the need to conduct 2-year studies in rats and mice of both sexes. Decisions could be based on a profile of data rather than on the result of one test. Procedures for regulatory acceptance of new approaches for carcinogenicity testing are critical to future progress.

Smith MT, Zhang L. **Biomarkers of leukemia risk: benzene as a model.** Environ Health Perspect 1998;106(Suppl 4):937-46.
Although relatively rare, leukemias place a considerable financial burden on society and cause psychologic trauma to many families. Leukemia is the most common cancer in children. The causes of leukemia in adults and children are largely unknown, but occupational and environmental factors are strongly suspected. Genetic predisposition may also play a major role. Our aim is to use molecular epidemiology and toxicology to find the cause of leukemia

and develop biomarkers of leukemia risk. We have studied benzene as a model chemical leukemogen, and we have identified risk factors for susceptibility to benzene toxicity. Numerous studies have associated exposure to benzene with increased levels of chromosome aberrations in circulating lymphocytes of exposed workers. Increased levels of chromosome aberrations have, in turn, been correlated with a heightened risk of cancer, especially for hematologic malignancy, in two recent cohort studies in Europe. Conventional chromosome analysis is laborious, however, and requires highly trained personnel. Further, it lacks statistical power, as only a small number of cells can be examined. The recently developed fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR)-based technologies have allowed the detection of specific chromosome aberrations. These techniques are far less time consuming and are more sensitive than classical chromosomal analysis. Because leukemias commonly show a variety of specific chromosome aberrations, detection of these aberrations by FISH and PCR in peripheral blood may provide improved biomarkers of leukemia risk.

Teeguarden JG, Dragan YP, Pitot HC. **Implications of hormesis on the bioassay and hazard assessment of chemical carcinogens.** Hum Exp Toxicol 1998;17(5):254-8.
Hormesis has been defined as a dose-response relationship which depicts improvement in some endpoint (increased metabolic rates, reduction in tumor incidence, etc.) at low doses of a toxic compound followed by a decline in the endpoint at higher doses. The existence of hormetic responses to carcinogenic agents has several implications for the bioassay and hazard assessment of carcinogens. To be capable of detecting and statistically testing for hormetic or other nonlinear dose-response functions, current study designs must be modified to include lower doses and sufficiently large numbers of animals. In addition, improved statistical methods for testing nonlinear dose-response relationships will have to be developed. Research integrating physiologically-based pharmacokinetic model descriptions of target dose with mechanistic data holds the greatest promise for improving the description of the dose-response curve at low doses. The 1996 Proposed Carcinogen Risk Assessment Guidelines encourage the use of mechanistic data to improve the descriptions of the dose-response curve at low doses, but do not distinguish between the types of nonlinear dose-response curves. Should this refined approach lead to substantial support for hormesis in carcinogenic processes, future guidelines will need to provide guidance on establishing safe doses and communicating the results to the public.

Tennant RW. **Evaluation and validation issues in the development of transgenic mouse carcinogenicity bioassays.** Environ Health Perspect 1998;106(Suppl 2):473-6.
Transgenic mouse models have emerged as plausible alternatives to long-term bioassays for carcinogenicity. Three transgenic lines evaluated to date have shown a clear capability to discriminate between carcinogens and noncarcinogens, using long-term bioassay results as the standard. The data also suggest that the transgenic lines will not fully duplicate long-term bioassay results. It is proposed that these models do not respond to chemicals that have induced highly restricted species or strain-specific tumor responses in mice or rats. Rather, the value of the transgenic models is predicated on a preferential response to transspecies carcinogens (i.e., those positive in both rats and mice, often including tumors in the same tissues). Thus, although results in transgenic models may not be completely concordant with long-term bioassays, the data can be used effectively in chemical and drug safety assessments. Further, it is proposed that validation of the models is readily achievable via ongoing studies. Validation of any alternative model is best achieved by sufficient mechanistic understanding of the model to reasonably predict the outcome of bioassays conducted in the models and use all available information on the drug or chemical. This goal can now be met with the transgenic mouse lines.

Van Deun K, Van Cauteren H, Vandenberghe J, Canning M, Vanparys P, Coussement W. **Review of alternative methods of carcinogenicity testing and evaluation of human pharmaceuticals.** Adverse Drug React Toxicol Rev 1997;16(4):215-33.
Hundreds of pharmaceuticals have been reported to give a positive result in the standard "Chronic Bioassay", which consists of an 18 to 24 month daily administration of the test compound in mice and rats. This is in contrast with 20 pharmaceuticals, which are known to be carcinogenic to humans. The high incidence of apparently false-positive results in the Chronic Bioassay may be related to differences in mechanism of pharmacological action between rodents and humans, but also to the very high dose levels that have to be administered to rodents in accordance to

regulatory guidelines. Lack of relevance to man therefore often has to be demonstrated by additional mechanistic studies. Based upon the deficiencies of the Chronic Bioassay and on the increased knowledge on cellular and molecular mechanisms involved in carcinogenicity, extensive discussions have recently taken place between regulatory agencies and industry associations at the occasion of International Conferences on Harmonization (ICH). These discussions have resulted in the possibility to use alternative short-to-medium-term carcinogenicity models in combination with a single two-year carcinogenicity study for evaluation of carcinogenicity. A description of these models is provided in this review as well as possible strategies for carcinogenicity testing and evaluation in the future.

Wilmer JL, Simeonova PP, Germolec DR, Luster MI. **Benzene and its principal metabolites modulate proinflammatory cytokines and growth factors in human epidermal keratinocyte cultures.** In Vitro Toxicol J Mol Cell Toxicol 1997;10(4):429-36.
A study was conducted examining the role of proinflammatory cytokines and growth factors in benzene (71432) induced skin carcinogenesis. The elaboration of interleukin-8 (IL-8) and tumor growth factor alpha (TGFa) by normal human skin keratinocytes in-vitro following exposure to benzene and its metabolic products was assessed. Secretion of TGFa and IL-8 increased following the addition of 1,4-benzosemiquinone (BQ), hydroquinone (123319) (HQ), or 1,2,4-benzenetriol (533733) (BZT) to skin cell cultures without concomitant cytotoxicity. Phenol (108952) increased the secretion of TGFa and IL-8 only at cytotoxic concentrations; IL-8 and TGFa secretion were not significantly affected by catechol (154234). Secretion of interleukin-6, tumor necrosis factor alpha, and MCP-1 was not significantly affected by BQ, HQ, or BZT. HQ induced production of IL-8 was significantly inhibited in the presence of N-acetylcysteine (NAC). BZT induced production of IL-8 was significantly inhibited by NAC as well as by dimethyl-sulfoxide and 1,1,3-tetramethylthiourea. Synergistic increases in IL-8 secretion were seen in incubations using a combination of CAT and HQ. A modest, but significant, increase in the production of IL-8 was seen following exposure to benzene. The authors conclude that exposure of human epidermal keratinocytes to relatively low concentrations of benzene or its metabolic products induces selective cytokines and growth factors. These agents may provide a mechanistic link between tumor initiation and promotion that will assist in the understanding of benzene induced skin carcinogenesis.

Wolfle D. **Interactions between 2,3,7,8-TCDD and PCBs as tumor promoters: limitations of TEFs.** Teratog Carcinog Mutagen 1997;17(4/5):217-24.
The interactive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (1746016) (TCDD) and the polychlorinated-biphenyls (PCBs) PCB126 (57465288) and PCB153 (35065271) on tumor promotion were examined. C3H/M2-mouse fibroblasts were cultured with the initiating agents N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 3-methylcholanthrene (MCA), or solvent control. Several days following pretreatment, the fibroblast cultures were exposed to varying concentrations and combinations of TCDD, PCB126, PCB153, or solvent control for up to 7 weeks. Cells were examined after 2 weeks to determine plating efficiency and after 8 weeks to determine the malignant transformation rate. The malignant transformation effects of MNNG and MCA were enhanced in cultures treated with 1.5x10(-13) molar (M) TCDD, 3x10(-13)M PCB126, or 3x10(-8)M PCB153. The transformation rate was highest in cultures exposed to TCDD plus PCB126 at a concentration of 3x10(-13)M. PCB126 and TCDD appeared to have an additive effect on cell transformation. In contrast, PCB153 inhibited the tumor promoting effects of TCDD in a dose dependent manner. PCB126 proved a more potent tumor promoter than PCB153. No malignant transformation was observed in cultures exposed to high concentrations of TCDD, PCB126, or PCB153 without pretreatment. This indicated that these compounds were not effective initiators in the malignant transformation assay. The plating efficiency of the fibroblasts was not affected by any of the treatment conditions. The author concludes that the tumor enhancing factor (TEF) approach may not be suitable for estimating the tumor promoting effects of polychlorinated hydrocarbons in animal tissues with high concentrations of diortho substituted PCBs.

CYTOTOXICITY

Bogaerts P, Senaud J, Bohatier J. **Bioassay technique using nonspecific esterase activities of Tetrahymena**

pyriformis for screening and assessing cytotoxicity of xenobiotics. Environ Toxicol Chem 1998;17(8):1600-5.
BIOSIS COPYRIGHT: BIOL ABS. A simple and rapid test for screening and assessing the cytotoxicity of xenobiotics was developed with Tetrahymena pyriformis. The method estimates the activities of nonspecific esterases of a cell by concentrating within it a specific amount of fluorescence associated with fluorescein dye. The 1-h median effective concentration (EC50) values of 10 inorganic and eight organic substances are presented and compared to those of three other bioassays: the conventional T. pyriformis proliferation rate 9-h median inhibitory concentrations, the Microtox 30-min EC50s, and the Daphnia magna 4-methylumbelliferyl beta-D galactoside 1-h EC50s. A highly significant correlation was found between the results obtained with the fluorescein diacetate test and those obtained with the growth inhibition and Microtox tests. This in vivo enzymatic test showed high sensitivity to all compounds tested except Cr6+ and sodium dodecyl sulfate.

Botsford JL. **A simple assay for toxic chemicals using a bacterial indicator.** World J Microbiol Biotechnol 1998;14(3):369-76.
BIOSIS COPYRIGHT: BIOL ABS. A simple, inexpensive and rapid technique to measure toxicity has been developed using Rhizobium meliloti as the indicator organism and its rapid reduction of the tetrazolium dye MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide). Toxic chemicals inhibited the reduction in this bacterium but not in others. Nearly 50 organic chemicals and 14 minerals were tested: inhibition was proportional to the concentration of the toxic chemical. The mechanism to account for both the reduction of the dye and the inhibition of reduction is uncertain. This method provides a simple and inexpensive way to determine the critical concentration of toxic compounds. The assay provides values comparable to those provided by the fat head minnow (Pimephales promelas). Results are also comparable to those obtained with the Micotox and Polytox assays, two commercial assays that use bacteria as indicator organisms.

Cordes N, Plasswilm L. **Cell line and schedule-dependent cytotoxicity of paclitaxel (Taxol): role of the solvent Cremophor EL/ethanol.** Anticancer Res 1998;18(3a):1851-7.
BACKGROUND: Paclitaxel's optimal dosage and scheduling is currently not determined. To compare paclitaxel (PTX) cytotoxicity in vitro, three cell lines were chosen for investigation by single versus fractionated exposure to Taxol and the diluent Cremophor EL/ethanol (CEL/eth). METHODS: An exponentially growing human lung-carcinoma (SK-LU-1), human glioblastoma (U-138 MG) and mammalian fibroblast cell line (HyB14FAF28) were used for colony forming assay examining cell survival, and flow cytometric DNA analysis by measuring cell cycle development. Tested concentrations varied from 2-50 microM and were incubated for 3 and 15 hours. Single (2-50 microM/d, especially 10 microM/d), versus fractionated (2 microM/d, day 1-5) exposure of Taxol and CEL/eth was investigated. As the control population, cells were exposed to a phosphate buffered solution (PBS). RESULTS: Control populations demonstrated an average survival of 90, 99 and 93% for SK-LU-1, U-138 MG, B14, respectively. Single Taxol exposure at 10 microM showed average survival of 54, 50 and 84% after 3 hours and 9, 48 and 82% after 15 hours for the above cell lines. Fractionated Taxol exposure with 2 microM/d, days 1-5 led to average survival of 55, 86 and 63%, respectively. Single CEL/eth exposure showed a cytotoxic effect with average survival of 94, 126 and 91% after 3 hours and 47, 63 and 88% after 15 hours respectively. Fractionated CEL/eth exposure showed an average survival of 67, 94 and 65% respectively. Flow cytometric analysis detected cell cycle shift concerning S- and G2/M-phase after Taxol exposure only in the two tumour cell lines, and not in the fibroblastic cells. CEL/eth was without significant effect on cell cycle distribution in all three cell lines. CONCLUSIONS: In the two human tumour cell lines cytotoxicity was more pronounced after prolonged Taxol exposure. The fibroblast cell line was not sensitive to single treatment, and was without cell cycle changes. Comparable to Taxol the diluent CEL/eth had a significant but less pronounced cytotoxic effect. Therefore, the cytotoxic mechanisms of paclitaxel's and CEL/eth's are worthy of further investigation.

Fladmark KE, Serres MH, Larsen NL, Yasumoto T, Aune T, Doskeland SO. **Sensitive detection of apoptogenic toxins in suspension cultures of rat and salmon hepatocytes.** Toxicon 1998;36(8):1101-14.
BIOSIS COPYRIGHT: BIOL ABS. A number of algal toxins were tested for the ability to induce apoptosis (regulated cell death) in primary hepatocytes from salmon and rat. The tested toxins included the liver targeting substances microcystin-LR and nodularin, substances associated with the diarrhetic shellfish poison complex (okadaic acid,

dinophysistoxin-1 and pectenotoxin-1) and calvculin A. All toxins induced apoptosis in both salmon and rat hepatocytes in less than 2 h. The apoptotic changes were evident both by electron and light microscopy and were counteracted by the caspase inhibitor ZVAD-fmk and by the Ca2+/calmodulin dependent kinase II inhibitor KN-93. The salmon hepatocytes were 10-20 fold more sensitive to okadaic acid and dinophysistoxin-1 (EC50 = 20 nM) than rat hepatocytes and other mammalian cell lines tested. An assay was devised using hepatocyte apoptosis as parameter for detection of algal toxins. This assay was at least as sensitive as HPLC determination for okadaic acid in mussel extracts. It also detected algal toxins which do not inhibit protein phosphatases, like pectenotoxin-1. Subapoptotic concentrations of the toxins inhibited hepatocyte aggregation. Using this parameter, less than 200 pg okadaic acid could be detected. In conclusion, salmon hepatocytes in suspension culture provide a rapid and sensitive system for detection of a broad range of apoptogenic toxins.

Geurtsen W, Lehmann F, Spahl W, Leyhausen G. **Cytotoxicity of 35 dental resin composite monomers/ additives in permanent 3T3 and three human primary fibroblast cultures.** J Biomed Mater Res 1998;41(3):474-80.
It was the purpose of this investigation to determine the cytotoxic effects (ED50 concentrations) of 35 monomers or additives identified in commercial dental resin composites. Monolayers of permanent 3T3 cells and three primary human fibroblast types derived from oral tissues (gingiva, pulp, and periodontal ligament) were used as test systems. All substances were tested in concentrations ranging from 0.01 to 5.0 mM. In general, ED50 values varied from 0.06 to > 5 mM. Within the groups of co(monomers), initiators, and cointiators, severe (e.g., Bis-GMA, UDMA, DMBZ, and DMDTA) or moderate (HEMA, BEMA, CQ, DMPT, and DMAPE) cytotoxic effects could be evaluated. Within the group of reaction/decomposition products, only moderate or slight effects were found (ED50: 0.7 to > 5 mM). The inhibitor BHT, the contaminant TPSb, and the photostabilizer HMBP, however, were highly cytotoxic in all cell cultures. In addition, the ED50 values of DBPO and HMBP significantly varied (0.43-3.8 mM, respectively, and 0.44-3.07 mM) with the applied cell culture. Our comprehensive screening shows that for several of the highly cytotoxic composite components, less cytotoxic alternatives are available. Furthermore, there was no cell type identified which was consistently less or more sensitive to the toxic effects of the tested compounds than the others. Primary human periodontal ligament and pulp fibroblasts, however, were found to be more sensitive than 3T3 and gingival fibroblasts to alterations from most tested substances.

Johnson SA, Dalton AE, Pardini RS. **Time-course of hypericin phototoxicity and effect on mitochondrial energies in EMT6 mouse mammary carcinoma cells.** Free Radical Biol Med 1998;25(2):144-52.
BIOSIS COPYRIGHT: BIOL ABS. Photoactivated hypericin produces singlet oxygen and superoxide anion radical; however, the intracellular events contributing to toxicity are unknown. Clonogenic assays of oxygen-dependent hypericin phototoxicity to EMT6 cells have previously shown that 0.5 muM hypericin + 1.5 J cm-2 fluorescent light is non-toxic and that 1.0 muM hypericin + 1.5 J cm-2 fluorescent light produces LD40 toxicity. Intracellular events leading to toxicity were revealed at these doses. Lactate dehydrogenase leakage was elevated for both 0.5 muM and 1.0 muM hypericin + light immediately following irradiation. While values eventually returned to control levels for 0.5 muM hypericin + light, leakage increased over time for 1.0 muM hypericin indicating reversible and irreversible toxicity, respectively. Increases in lipid and protein oxidation were measured immediately following irradiation; however, these parameters return to control levels within 0.5 h for both doses. Both total cellular ATP levels and cellular respiration were depressed by approximately 50% of control values for 1.0 muM hypericin + light. These values were unchanged for 0.5 muM hypericin + light. Along with previously reported data demonstrating that light-activated hypericin can inhibit mitochondrial succinoxidase in beef heart mitochondria in vitro, these data support oxidative stress-initiated mitochondrial damage as a key target in hypericin phototoxicity.

Keese CR, Karra N, Dillon B, Goldberg AM, Giaever I. **Cell-substratum interactions as a predictor of cytotoxicity.** In Vitro Mol Toxicol 1998;11(2):183-92.
BIOSIS COPYRIGHT: BIOL ABS. An in vitro toxicity assay based on an emerging biosensor technology referred to as ECIS (electric cell-substrate impedance sensing) has been developed and tested. In ECIS, adherent cells are cultured on small gold electrodes and electrical impedance is monitored with a computer-interfaced instrument Subtle changes in cell morphology and behavior including cell motility can be detected with this approach. Confluent

cell monolayers (WI38/VA13 fibroblastic and MDCK epithelial cells) were exposed to varying concentrations of four detergents (Tween 20, benzalkonium chloride, Triton X100, and sodium lauryl sulfate). ECIS measurements were used to follow subsequent changes of the overall impedance of the cell monolayer and of cell motions detected as impedance fluctuations. Analysis of these measurements can correctly rank the detergents according to their established in vivo toxicity. Of particular interest was the dramatic increase of impedance fluctuations sometimes recorded from cells upon exposure to the toxicants. This occurred at times and detergent concentrations below those showing a decline in overall impedance; the effect was particularly evident in the MDCK cells.

Kitasaka H, Oshima H, Nakamura M. **[In vitro induction of stress protein by chemicals in HeLa cells].** Shika Igaku 1997;60(4):273-87. (Jpn)
BIOSIS COPYRIGHT: BIOL ABS. We evaluated the cellular reactions to ten chemicals including components of dental cements by examining stress protein synthesis and cell viability with neutral red (NR) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Induction of 70,000-Da stress protein (HSP 70) synthesis in HeLa cells by chemicals was evaluated by an enhanced chemiluminescence system. HSP 70 synthesis was induced during the early stage between three and six hours of exposure to phosphoric acid, methyl methacrylate monomer (MMA), formalin and hydrochloric acid at concentrations of 50 to 500 muM. Acrylamide induced HSP 70 to a small extent, while the other agents examined, i.e. eugenol, itaconic acid, chloroform, acetone and xylene, had no effect. The results of NR and MTT assays revealed that phosphoric acid, eugenol, itaconic acid, formalin, hydrochloric acid and acrylamide at concentrations of 500 muM and over, inhibited the cells, while MMA, chloroform, acetone and xylene did not. It was concluded that the synthesis of HSP 70 might represent a cellular change caused by acidic chemicals, and that the concentration that induced HSP 70 synthesis was lower than that inhibiting cell viability.

Kohlhagen G, Paull KD, Cushman M, Nagafuji P, Pommier Y. **Protein-linked DNA strand breaks induced by NSC 314622, a novel noncamptothecin topoisomerase I poison.** Mol Pharmacol 1998;54(1):50-8.
NSC 314622 was found to have a cytotoxicity profile comparable to the topoisomerase I (top1) inhibitors camptothecin (CPT) and saintopin in the National Cancer Institute In Vitro Anticancer Drug Discovery Screen using the COMPARE analysis. In vitro data showed that NSC 314622 induced DNA cleavage in the presence of top1 at micromolar concentrations. Cleavage specificity was different from CPT in that NSC 314622 did not cleave all sites induced by CPT whereas some sites were unique to the NSC 314622 treatment. Top1-induced DNA cleavage was also more stable than cleavage induced by CPT. NSC 314622 did not induce DNA cleavage in the presence of human topoisomerase II. High concentrations of NSC 314622 did not produce detectable DNA unwinding, which suggests that NSC 314622 is not a DNA intercalator. DNA damage analyzed in human breast carcinoma MCF7 cells by alkaline elution showed that NSC 314622 induced protein-linked DNA single-strand breaks that reversed more slowly than CPT-induced strand breaks. CEM/C2, a CPT-resistant cell line because of a top1 point mutation [Cancer Res 55:1339-1346 (1995)], was cross-resistant to NSC 314622. These results demonstrate that NSC 314622 is a novel top1-targeted drug with a unique chemical structure.

Krzyzaniak JF, Alvarez Nunez FA, Raymond DM, Yalkowsky SH. **Lysis of human red blood cells. Part 4. Comparison of in vitro and in vivo hemolysis data.** J Pharm Sci 1997 Nov;86:1215-7.
IPA COPYRIGHT: ASHP A dynamic in vitro method and other in vitro methods were used to determine the degree of hemolysis induced by several cosolvent vehicles used for parenteral formulations that have previously been evaluated in vivo; the in vitro data generated for each of the vehicles was compared with the in vivo hemolysis data to assess the ability of the method to estimate in vivo hemolysis. Results showed that the in vitro data generated by the dynamic method are in agreement with the in vivo data for each vehicle. Therefore, the potential for formulations to induce intravascular hemolysis after injection can be determined by this method.

Lerza R, Castello G, Ballarino P, Mela GS, Tredici S, Arboscello E, Mencoboni M, Pannacciulli I. **In vitro toxicity of A 3'-azido-3'-deoxythymidine and hydroxyurea combination on normal myeloid progenitors.** Anticancer Res 1998;18(4a):2755-8.

Hydroxyurea (HU) appears to increase 3'-azido-3'-deoxythymidine (AZT) antiretroviral activity and cytotoxicity by inhibiting thymidilate synthesis. The combination of AZT and HU may therefore be of clinical usefulness. We evaluated the in vitro hemotoxicities of different combinations of AZT and HU in comparison with the hemotoxicities exerted by either of the two drugs alone. Peripheral blood granulocyte macrophage committed progenitors (CFU-GM) of healthy donors were selected as targets of hemotoxicity. Both AZT and HU separately had a dose-dependent inhibitory effect on the in vitro growth of normal circulating CFU-GM. The combination of the two drugs induced a statistically significant synergistic cytotoxicity. In fact, addition of HU induced a remarkable reduction of AZT ID50. Thus, future clinical application of AZT, HU combination should take into account the greater hemosuppressive action of the combination in respect to that observed following administration of either drug alone.

MacDougall M, Selden JK, Nydegger JR, Carnes DL. **Immortalized mouse odontoblast cell line MO6-G3 application for in vitro biocompatibility testing.** Am J Dent 1998;11(Spec No.):11-6.
PURPOSE: This study was designed to determine the usefulness of an established stable immortalized mouse odontoblast cell line (MO6-G3) for dental material biocompatibility testing. Using a standard toxicity assay based on cell respiratory activity, the response to MO6-G3 cells was compared to the mouse fibroblastic cell line, L929, presently used for dental materials testing. The dental resin monomer TEGDMA was used as the dental material for the assay. MATERIALS AND METHODS: Cell lines (1 x 10(3)/well) were plated in 96 well culture plates and grown in DMEM supplemented with 10% FCS, 100 units/ml each of penicillin and streptomycin, and 50 micrograms/ml ascorbic acid in an atmosphere of 95% air and 5% CO2. Cells were exposed to TEGDMA resin monomer covering a dose range of 1 x 10(-6) to 0.5 x 10(-3) M. Unexposed control cells, as well as cells exposed to the DMSO vehicle in which the TEGDMA was dissolved, were included in all assays. Cytotoxicity was evaluated by determining cell respiratory activity spectrophotometrically using the tetrazolium compound WST-1. RESULTS: Statistical analysis by ANOVA using Tukey's method for pair wise comparisons as the post hoc test indicated toxic effects of TEGDMA at 1 x 10(-5) M in the odontoblast cell line MO6-G3. By contrast, the monomer produced no toxic effects on the L929 fibroblast cell line after 24 hours of exposure, over the entire concentration range tested. Furthermore, MO6-G3 cells exposed to a concentration of 0.5 x 10(-3) M were unable to recover from the effects of the exposure 48 hours after removal of the resin. MO6-G3 cells exposed to 1 x 10(-4) and 0.5 x 10(-4) TEGDMA recovered 40-50% and 75-80% of control respiratory activity respectively, 48 hours after removal of the resin. Respiratory activity by L929 cells exposed to all TEGDMA concentrations tested was not different from the vehicle control 48 hours after removal of the resin.

Mamdouh Z, Giocondi MC, Le Grimellec C. **In situ determination of intracellular membrane physical state heterogeneity in renal epithelial cells using fluorescence ratio microscopy.** Eur Biophys J 1998;27(4):341-51.
6-Lauroyl-2-dimethylaminonaphtalene (laurdan) shows a spectral sensitivity to the lipid phase state with a 50 nm red shift of the emission maximum when passing from the gel to the liquid crystalline phase. This spectral sensitivity allows one to determine the membrane physical state using Generalized Polarization (GP). In the present experiments, we used fluorescence ratio imaging microscopy to determine the laurdan GP in living kidney cells. Two renal epithelial cells lines, MDCK and LLC-PK1 cells, and CV-1 cells, a fibroblast-like renal cell line were investigated. In these cells, laurdan labels both the plasma membrane and intracellular membranes. Comparison of spectrofluorimetry and fluorescence ratio imaging data obtained from liposomes and cells suspensions labeled with laurdan demonstrates that the GP can be accurately determined using common fluorescence microscopy equipment. The GP mean values determined from individual cells varied from 0.2 to 0.4 for the epithelial cells as compared to 0.0-0.1 for CV1 cells. Using living MDCK cells grown as a monolayer, the GP maps indicated that, within a single cell, the intracellular GP values varied from 0.0 to 0.6, i.e., from the equivalent of a liquid-crystalline state to a gel or a lipid-ordered state, and that there was a marked heterogeneity in the spatial distribution of the GP values. To further characterize this intracellular heterogeneity, co-localization experiments with specific organelle markers were undertaken. The results strongly suggest that in intact cells at physiological temperature, GP values decrease in the following order: plasma membranes > endosomes > mitochondria > Golgi apparatus.

Mesa-Valle CM, Rodriguez-Cabezas MN, Moraleda-Lindez V, Craciunescu D, Sanchez-Moreno M, Osuna A. **In vitro and in vivo activity of two Pt(IV) salts against Leishmania donovani.** Pharmacology 1998;57(3):160-72.

The activities of 8 platinum drug complex salts were determined against Leishmania donovani promastigotes. The three most active salts were selected: [PtIVBr6]H2 (pentamidine); [PtIVBr6]H2 (stilbamidine), and [PtIVCl6]H2 (2-piperazinyl(1) ethyl amine), which induced growth-inhibition rates of more than 50% at 24 h of treatment and at the maximum dosage tested. The cytotoxicity assays on the macrophage cell line J-774 showed high cytotoxicity for the salt [PtIVBr6]H2 (stilbamidine) with a percentage of specific 51Cr release of 58.2% at 24 h of incubation and 100 microg/ml. Meanwhile, assays of the other compounds showed practically no cytotoxicity. The salt [PtIVBr6]H2 (pentamidine) notably inhibited the incorporation of 3H-thymidine in the treated parasites. The ultrastructural alterations observed in the flagellates treated with the salts [PtIVCl6]H2 (2-piperazinyl(1)ethyl amine) and [PtIVBr6] H2 (pentamidine) suggest that both act preferentially at the nuclear level and at the kinetoplast-mitochondrion complex. Both compounds showed a high in vivo activity in parasitized Wistar rats.

Nakagawa H, Murata M, Tachibana K, Shiba T. **Screening of epiphytic dinoflagellates for radical scavenging and cytotoxic activities.** Physiol Res 1998;46(Suppl):9-12.
BIOSIS COPYRIGHT: BIOL ABS. In the search for antioxidant substances/radical scavengers and cytotoxic substances, extracts from four species (10 strains) of epiphytic dinoflagellates in laboratory cultures were subjected to screening for production of bioactive metabolites. Assays for antioxidants were performed using microsomal lipids prepared from rat livers, which were oxidized with Fe3+-nitrilotriacetic acid complex (Fe3+-NTA). Generated lipid peroxides were determined using the thiobarbituric acid (TBA) colorimetric method. Screening tests for cytotoxic activity were carried out using P388 leukemic cells of which the survival ratio was assessed using the tetrazolium salt (MTT) method. Extracts from two strains (Gymnodinium sp. and Gambierdiscus toxicus) and those from five strains (two of G. toxicus, two of Coolia monotis, and one of Prorocentrum sp.) were found to contain active constituents for antioxidant activity and for cytotoxic activity, respectively.

Naylor MA, Swann E, Everett SA, Jaffar M, Nolan J, Robertson N, Lockyer SD, Patel KB, Dennis MF, Stratford MR, et al. **Indolequinone antitumor agents: reductive activation and elimination from (5-methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl derivatives and hypoxia-selective cytotoxicity in vitro.** J Med Chem 1998;41(15):2720-31.
A series of indolequinones bearing a variety of leaving groups at the (indol-3-yl)methyl position was synthesized by functionalization of the corresponding 3-(hydroxymethyl)indolequinone, and the resulting compounds were evaluated in vitro as bioreductively activated cytotoxins. The elimination of a range of functional groups-carboxylate, phenol, and thiol-was demonstrated upon reductive activation under both chemical and quantitative radiolytic conditions. Only those compounds which eliminated such groups under both sets of conditions exhibited significant hypoxia selectivity, with anoxic:oxic toxicity ratios in the range 10-200. With the exception of the 3-hydroxymethyl derivative, radiolytic generation of semiquinone radicals and HPLC analysis indicated that efficient elimination of the leaving group occurred following one-electron reduction of the parent compound. The active species in leaving group elimination was predominantly the hydroquinone rather than the semiquinone radical. The resulting iminium derivative acted as an alkylating agent and was efficiently trapped by added thiol following chemical reduction and by either water or 2-propanol following radiolytic reduction. A chain reaction in the radical-initiated reduction of these indolequinones (not seen in a simpler benzoquinone) in the presence of a hydrogen donor (2-propanol) was observed. Compounds that were unsubstituted at C-2 were found to be up to 300 times more potent as cytotoxins than their 2-alkyl-substituted analogues in V79-379A cells, but with lower hypoxic cytotoxicity ratios.

Neal GE, Eaton DL, Judah DJ, Verma A. **Metabolism and toxicity of aflatoxins M1 and B1 in human-derived in vitro systems.** Toxicol Appl Pharmacol 1998;151(1):152-8.
Aflatoxin M1 (AFM1) is the principal hydroxylated aflatoxin metabolite present in the milk of dairy cows fed a diet contaminated with aflatoxin B1, (AFB1) and the metabolite is also present in the milk of human nursing mothers consuming foodstuffs containing the toxin. AFM1 is usually considered to be a detoxification product of AFB1 and this appears warranted if the biological endpoints involved are carcinogenicity and mutagenicity. However, it may not be a valid conclusion in the case of cytotoxicity. The metabolism of AFM1 and AFB1 have been studied in vitro using human liver microsomes. Formation of primary metabolites associated with metabolic activation to the respective epoxides reflected the differences between the carcinogenic potentials of the two toxins and, similar to

AFB1, the conjugation of AFM1 epoxide with reduced GSH was catalyzed by mouse, but not human liver cytosol. Although the majority of the binding of [3H]AFB1 to microsomal protein was dependent on metabolic activation, a high level of retention of [3H]AFM1 by microsomes, nonextractable in methanol and unrelated to metabolic activation, was observed. It appears possible that this property is related to the high cytotoxicity of AFM1. Experiments using human cell line cells either expressing or not expressing human cytochrome P450 enzymes in assays of acute toxicity (MTT assays) have demonstrated a directly toxic potential of AFM1 in the absence of metabolic activation, in contrast to AFB1. Caution therefore needs to be exercised in designating the formation of AFM1 as essentially detoxification when considering a biological response in which cytotoxicity may play a significant role, e.g., immunotoxicity. Copyright 1998 Academic Press.

Nociari MM, Shalev A, Benias P, Russo C. **A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity.** J Immunol Methods 1998;213(2):157-67.
In this study, a fluorometric method using alamarBlue has been developed for detecting cell-mediated cytotoxicity in vitro. AlamarBlue is a non-toxic metabolic indicator of viable cells that becomes fluorescent upon mitochondrial reduction. Specific lysis of targets by effector cells is quantified by comparing the total number of viable cells in wells containing effector and targets together, with wells where target and effector cells were separately seeded. Cell-mediated cytotoxic activity by alloreactive T cells and natural killer cells has been detected using a novel application of the alamarBlue technique. The assay that we have developed to detect cell-mediated cytotoxicity is extremely sensitive and specific and requires a significant lower number of effector cells than the standard 51Cr assay. Since alamarBlue reagent is non-toxic to cells and the assay can be performed under sterile conditions, effector cells may be recovered at the end for further analysis or cell expansion, if desired. Direct comparison of cell-mediated cytotoxicity measured by the alamarBlue method with the standard 51Cr release assay revealed that the former method is as specific and more sensitive than the conventional assay. Moreover, very small inter and intra-assay variations have been observed for alamarBlue cytotoxicity assays. In conclusion, this study shows that the alamarBlue assay is an extremely sensitive, economical, simple and non-toxic procedure to evaluate cell-mediated cytotoxicity that yields accurate results using a limited number of effector cells. Furthermore, since this assay is a one-step procedure, and does not involve any risk for the personnel, it may be useful to analyze automatically cell-mediated cytotoxicity in a large number of samples.

Ohba H, Nishikawa M, Kimura M, Yamasaki N, Moriwaki S, Itoh K. **Cytotoxicity induced by Erythrina variegata serine proteinase inhibitors in tumor hematopoietic stem cell lines.** Biosci Biotechnol Biochem 1998;62 (6):1166-70.
Based on the soluble MTT tetrazolium/formazan assay, we evaluated the cytotoxicity of Erythrina variegata proteinase inhibitors in some tumor hematopoietic stem cell lines. Among the proteinase inhibitors, EBI, which belongs to the Bowman-Birk family of inhibitors, was cytotoxic in relatively differentiated cells such as Molt4 and Jurkat derived from acute T lymphoblastic leukemia (T-ALL) cells specifically, but ETIa and ECI, which are classified into Kunitz family inhibitors, did not. It was suggested that the differences in the cytotoxicity might be due to the molecular size of the inhibitors. The succinylation of lysine residue(s) of EBI led to about 50% loss of the trypsin inhibitory activity as compared with the authentic EBI. When Molt4 cells were incubated with this derivative, no significant cytotoxicity was observed. This suggests that the proteinase inhibitory activity might be involved in the cytotoxicity in human tumor cell lines.

Page B, Renaud N, Page M. **A non isotopic method for the measurement of cell membrane integrity.** Anticancer Res 1998;18(4a):2313-6.
In vitro cytotoxic assays often use various technical approaches for the measurement of cell mortality. Assays such as trypan blue exclusion or chromium release measure cell membrane integrity but they are either time consuming, not sensitive enough or they generate radioactive wastes. We have developed a dehydrogenase release assay which takes advantage of a new fluorescent amplification system. Mouse L929 fibroblasts and tumor necrosis factor (TNF-alpha) were used as a model system. This approach is sensitive, rapid, reproducible and may be used advantageously for mixed lymphocyte reaction (MLR). This new economical assay may be easily automated for large scale cytotoxicity testing using cell membrane integrity.

Roepstorff V, Sigsgaard T. **Cytotoxic effect of organic dust extracts from different working environments: an in vitro assay.** Ann Agric Environ Med 1997;4(2):195-201.
An assay for detecting cytotoxicity of organic dust extracts was developed. Monkey kidney VERO cells and human lung carcinoma A549 cells cultured in microtiter plates at the rate of 5x10(3) cells per well were treated with 0.48 and 15.6 milligrams per well of aqueous or alkaline (pH 8.3) extracts of each test dust. Test dusts were prepared from crude cotton dust, grain dust, swine confinement dust, compacted waste dust from a garbage handling facility, and household garbage compost dust. The medium containing the dust extracts was then removed and replaced with medium containing 15% of an unspecified tetrazolium salt dye. After 4 hours (hr), a solubilizer was added and the cells were incubated overnight. The absorbance of the cultures was then measured at 570 nanometers (nm) using an enzyme linked immunosorbent assay (ELISA) microplate reader. Cytotoxicity of each test dust was assessed by measuring decreases in the ability of the cells to reduce the tetrazolium salt to formazan-blue, the concentration of which was determined from the absorbance measurements. The dust extracts demonstrated greater cytotoxicity to VERO cells than to A549 cells. The alkaline extracts generally produced significantly greater cytotoxic effects than the aqueous extracts. The compost and grain dust extracts induced the greatest cytotoxicity, significant responses being seen after 2hr of incubation. The authors conclude that the assay using both cell types is a useful tool for determining the cytotoxicity of organic dusts found in different work environments.

Ruiz MJ, Marzin D. **Genotoxicity of six pesticides by Salmonella Mutagenicity Test and SOS Chromotest.** Mutat Res 1997;390(3):245-55.
The genotoxic activity of atrazine (1912249), captafol (2425061), captan (133062), chlorpyrifos-methyl (5598130), molinate (2212671), and tetrachlorvinphos (22248799) was examined using two in-vitro tests, the Ames test and the SOS chromotest. Salmonella-typhimurium strains TA-1535, TA-1537, TA-98, TA-100, and TA-102 were cultured and exposed to pesticide doses varying from 0 to 1,000 micrograms per plate (microg/plate) in the presence or absence of S9 fraction. The number of revertants per plate was determined. The Escherichia-coli strain PQ37 was incubated and exposed to pesticide doses ranging from 0 to 1,000microg/plate in the presence or absence of S9 fraction. The measured beta-galactosidase and alkaline-phosphatase activities were used to calculate the induction factor (IF) of each pesticide. Captafol was not mutagenic in Salmonella strains TA-1535, TA-1537, and TA-98 and was possibly mutagenic in the TA-100 strain. Captafol was definitely mutagenic in the TA-102 strain at concentrations of 1.25 to 10microg/plate without metabolic activation and 0.5 to 50microg/plate with metabolic activation. Captan exhibited dose dependent mutagenic activity in all of the Salmonella strains tested with or without metabolic activation. The presence of the S9 fraction increased the mutagenic effects of captan in the TA-1535, TA1537, TA-98, and TA-102 strains. Both captafol and captan were also genotoxic in E-coli in both the presence and absence of metabolic activation. The IF values were higher for captafol than for captan. Genotoxic activity was not demonstrated by atrazine, chlorpyrifosmethyl, tetrachlorvinphos, or molinate in either of the assays. The authors conclude that the use of the Ames test in conjunction with the SOS chromotest may be applied to the determination of the carcinogenotoxicity of pesticides.

Streffer C, Muller WU, Kryscio A, Bocker W. **Micronuclei-biological indicator for retrospective dosimetry after exposure to ionizing radiation.** Mutat Res 1998;404(1-2):101-5.
Micronuclei can be measured through a conventional method after staining with Giemsa or fluorescence dyes for DNA. However, a technique with cell proliferation control should be preferred. This is done by incubation with cytochalasin B and counting the micronuclei in binucleated cells. Satisfactory dose relationships are observed after irradiation of human lymphocytes in vitro. The RBE for fast neutrons is around three. An automatic analysis is possible by image analysis. The dose range in which significant increases can be observed is 0.3 to 5 Gy X-rays. The assay becomes more sensitive when the micronuclei are determined only in B-lymphocytes. Another possibility exists by determination of the number of micronuclei with centromeres. For this purpose the hybridization with pancentromeric DNA probes and fluorescence labelling is of advantage. By this technique a radiation dose of 0.1 Gy X-rays can be detected. It is apparently also possible under these conditions to detect radiation exposures which have taken place decades before the measurements. Copyright 1998 Elsevier Science B.V.

Villaescusa I, Matas C, Hosta C, Martinez M, Murat JC. **Evaluation of lead(III) and nickel(II) toxicity in NaCl and NaClO4 solutions by using Microtox bioassay.** Fresenius' J Anal Chem 1998;361(4):355-8.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity of nickel and lead compounds has been evaluated in NaCl and NaClO4 solutions by using the MicrotoxR bioassay. The aim of this work was to assess the toxicity of the different species in solution when varying the concentration of the medium. A modified Microtox protocol which enables the pH to be constant in all the cuvettes during analysis has been used. The positively charged species (Ni2+, Pb2+, PbCl+) were found to contribute more to the toxicity of Ni and Pb in NaCl while in NaClO4 solutions free metal was the responsible species. In all media studied, Pb compounds were the most toxic and provoked a quick response of the bacteria.

Wittig A, Sauerwein W, Poller F, Fuhrmann C, Hideghety K, Streffer C. **Evaluation of boron neutron capture effects in cell culture using sulforhodamine-B assay and a colony assay.** Int J Radiat Biol 1998;73(6):679-90.
PURPOSE: The purpose of this study was to find an in vitro method for determining the cytotoxicity of boronated drugs as well as their potential suitability for neutron capture therapy. MATERIALS AND METHODS: The survival of human melanoma cells has been determined by a colony assay and the sulforhodamine-B assay after X-irradiation and irradiation with fast d(14) + Be-neutrons using the boronated compound borocaptate sodium (BSH). The cytotoxic effects of BSH have been studied using both methods. RESULTS: Under well-defined experimental conditions, and after a sufficient amount of time for the expression of radiation damage, the results of the sulforhodamine-B assay are qualitatively comparable with the results of the colony assay. CONCLUSION: The sulforhodamine-B assay is suitable for the screening of compounds for potential use in neutron capture therapy because it is a fast and efficient method that is reproducible and technically advantageous.

DERMAL TOXICITY

Ali A, Radha S, Agarawal SP. **Fabrication of a diffusion cell for the determination of drug release from topical aerosol formulations.** Indian Drugs 1997 Dec;34:715-7.
IPA COPYRIGHT: ASHP The construction of a diffusion cell for use in the evaluation of the skin permeation of drugs from topical aerosol formulations is described; the diffusion cell was used to study the permeation of piroxicam from topical aerosol formulations containing 1.2% of the drug and 0.5, 1, 2, or 3% polyethylene glycol 400 (PEG 400) through excised rat abdominal skin. The diffusion cell apparatus worked well in the evaluation of the formulations of piroxicam. The in vitro permeation of piroxicam through rat skin increased as the concentration of polyethylene glycol 400 in the formulations increased.

Arra GS, Arutla S, Krishna DR. **Transdermal delivery of isosorbide 5-mononitrate from a new membrane reservoir and matrix-type patches.** Drug Dev Ind Pharm 1998;24(5):489-92.
IPA COPYRIGHT: ASHP To prepare transdermal isosorbide mononitrate (isosorbide 5-mononitrate) patches, 500 mg polyvinyl alcohol matrices containing 500 mg isosorbide mononitrate and polymeric membranes containing isosorbide mononitrate and a film-forming material extracted from the roots of Salacia macrosperma were prepared and evaluated for skin permeation in vitro. The mean flux values from the matrix and reservoir patches were 99.55 and 31.82 mcg/sq cmh, respectively.

Barratt MD, Brantom PG, Fentem JH, Gerner I, Walker AP, Worth AP. **The ECVAM International Validation Study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals.** Toxicol In Vitro 1998;12(4):471-82.
BIOSIS COPYRIGHT: BIOL ABS. An international validation study on in vitro tests for skin corrosivity was conducted during 1996 and 1997 under the auspices of the European Centre for the Validation of Alternative Methods (ECVAM). The main objectives of the study were to assess the performances of selected in vitro tests in discriminating between: (a) corrosives (C) and non-corrosives (NC), for selected groups of chemicals (e.g. organic acids, phenols) and/or for all chemicals (single chemical entities only); and (b) known R35 (UN packing group I) and

R34 (UN packing groups II & III) chemicals. Each test was evaluated for reliability and relevance by using a test set of 60 coded chemicals. In this paper, the test chemicals used in the validation study are identified; they include organic acids (6C/5NC), organic bases (7C/3NC), neutral organics (9NC), phenols (2C/3NC), inorganic acids (6C/1NC), inorganic bases (2C/2NC), inorganic salts (IC/2NC), electrophiles (3C/5NC) and soaps/surfactants (3NC). The in vivo classifications and important physicochemical properties (e.g. logP, pKa) of the test chemicals are given. The main criterion for including chemicals in the test set was that their corrosivity classifications were based on unequivocal animal data. Where available, structure-activity information was also used to support the corrosivity classifications. Despite the small numbers of chemicals in some of the categories, it was felt that the test set chosen represented the best possible for evaluating the performances of the in vitro tests for predicting skin corrosivity, given the limited availability of unequivocal animal data. The prediction of skin corrosivity from pH data was also investigated for those chemicals with extreme pH values (i.e. pH : 2 or : 11.5). Nine of the 12 strongly acidic or alkaline chemicals in the test set, which were predicted to be C on the basis of their pH values, had also been found to be C in vivo.

Basketter DA. **Chemistry of contact allergens and irritants.** Am J Contact Dermat 1998;9(2):119-24.
Understanding the nature and causes of contact dermatitis is as much a problem of chemistry as it is of biology. Many of the characteristics of an individual's reaction to a particular chemical, whether it be an irritant or allergen or both, depend very much on that person. From a more general perspective, however, the ability of a chemical to irritate and/or sensitize is a primary function of its chemical properties. The human biological system simply responds to the nature of the chemical perturbation it perceives; thus within the human population there is normal distribution in terms of the intensity of a reaction to a defined stimulus. This article reviews the present state of knowledge regarding the relationships between the ability of a chemical to cause contact dermatitis and its physicochemical properties, with close attention paid to how this knowledge may be used in a predictive sense to assist in risk assessment.

Basketter DA, Gerberick GF, Kimber I. **Strategies for identifying false positive responses in predictive skin sensitization tests.** Food Chem Toxicol 1998;36(4):327-33.
It is important that predictive toxicological test methods are selective for their intended endpoint and that their limitations are understood and acknowledged. The local lymph node assay (LLNA) is a relatively new predictive test for skin sensitization potential that can replace traditional guinea pig tests and offers significant scientific and animal welfare advantages. However, there has been some concern that certain irritant materials may yield false positive results, although it must be emphasized that false positives also occur in guinea pig methods. Consequently, we have examined the performance in the LLNA of a range of skin irritants, from varying chemical classes and covering a range of irritation potency. The results presented here demonstrate clearly that the majority of skin irritants are negative in the LLNA. These results are reviewed in the context of the occurrence of false positive reactions in the guinea pig maximization test and the strategies for dealing with such results are discussed. The need for careful scientific evaluation of the results in all predictive tests for sensitization is thus emphasized. In terms of specificity, the LLNA has been more fully evaluated than other predictive test methods and is at least as accurate. In terms of animal welfare, objectivity, reproducibility and reliability it is superior to other methods. In summary, all predictive skin sensitization test results should be evaluated in a scientifically rigorous manner and the additional data provided herein further support the adoption of the LLNA as a complete replacement for the traditional guinea pig methods.

Basketter DA, Miettinen J, Lahti A. **Acute irritant reactivity to sodium lauryl sulfate in atopics and non-atopics.** Contact Dermatitis 1998;38(5):253-7.
Predictive testing of chemicals to assess their acute skin irritation potential is an important part of the assessment of their toxicological profile. It is possible, where safety and ethical considerations can be met, to do this work in groups of human volunteers. Previously, the relative responsiveness of atopics and non-atopics has been evaluated. The results showed that atopics (defined broadly by high IgE reactivity) were a little more susceptible to skin irritation, but not significantly so. In the present work, the relative reactivity of a skin atopic group versus a non-atopic group was examined in more detail. Sodium lauryl sulfate (SLS) was applied at a range of concentrations

and exposure times, such that a fairly constant degree of skin irritation was produced. At various time points, the irritation response was measured by visual assessment, chromametry, laser Doppler flowmetry and transepidermal water loss. Using all of the methods of assessment, the reactions in atopics were similar to or a little less than those seen in non-atopics. The conclusion is that atopics and non-atopics will give similar results in a predictive human test for acute skin irritation. Furthermore, the pattern of response obtained from short duration exposure should be predictive of that following longer durations of (single) exposure.

Baynes RE, Halling KB, Riviere JE. **The influence of diethyl-m-toluamide (DEET) on the percutaneous absorption of permethrin and carbaryl.** Toxicol Appl Pharmacol 1997;144(2):332-9.
The effect of diethyl-m-toluamide (134623) (DEET) on the percutaneous absorption of permethrin (52645531) and carbaryl (63252) was examined, in order to investigate the possibility that enhanced absorption of pesticides with simultaneous dermal exposure to DEET might be associated with Gulf War Syndrome. Aqueous dosing solutions containing permethrin or carbon-14 (C14) labeled carbaryl at concentrations equivalent to 500 and 40 micrograms per square centimeter, respectively, and 0 or 15 or 35% DEET and 40 or 80% acetone (67641), ethanol (64175), or dimethyl-sulfoxide (67685) as vehicle were applied to discs prepared from the dorsal skin of male C3H-mice, albino-rats, or weanling female Yorkshire-pigs mounted on flow through diffusion cells. Perfusate samples were collected hourly for 8 hours (hr) in the rat and pig skin experiments and at 2, 4, 6, 8, 12, 16, and 24hr in the mouse skin experiments. The skin samples were analyzed for carbaryl or permethrin residues. No permethrin was absorbed by rat or pig skin within 8hr or by mouse skin within 24hr when treated with the permethrin plus DEET solutions regardless of the solvent used. Small amounts of permethrin were absorbed when permethrin was applied alone in acetone and DMSO, 1.7 and 1.2% of the dose, respectively. Carbaryl alone was readily absorbed through pig skin when acetone or DMSO was used as the solvents, the maximum absorption being 9.46% in 40% acetone and 2.94% in 40% DMSO, respectively. DEET inhibited carbaryl absorption when applied in acetone. DEET did not enhance carbaryl absorption when applied to pig skin in DMSO, but significantly increased the extent of carbaryl penetration into the stratum corneum. When applied in 40% acetone, significant retention of carbaryl on the skin surface was also found. DEET readily penetrated skin samples from all species, 10.7 to 20.6% of the dose being absorbed by mouse skin, 1.1 to 5.2% by rat skin, and 2.8% being absorbed by pig skin. When applied to rat and mouse skin, maximal DEET absorption occurred when DMSO and acetone were used as solvents. The authors conclude that DEET, but not permethrin or carbaryl, can be absorbed in sufficient quantities to cause systemic toxicity. DEET does not necessarily enhance dermal absorption of pesticides or other toxicants.

Bhatia KS, Gao S, Freeman TP, Singh J. **Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin.** J Pharm Sci 1997 Sep;86:1011-15.
IPA COPYRIGHT: ASHP The effects of 2 different chemical penetration enhancers, ethyl alcohol (absolute ethanol) and a preparation of 10% oleic acid in ethyl alcohol, and iontophoresis on the in vitro permeability of the radiolabeled peptide sincalide (cholecystokinin-8) through porcine epidermis and on the ultrastructural changes in stratum corneum were investigated. Pretreatment with the penetration enhancers significantly increased the permeability coefficient of sincalide in comparison with a control (pretreated epidermis without penetration enhancer). Iontophoresis further increased the permeability of sincalide through penetration enhancer pretreated epidermis in comparison with the control. The ultrastructure of stratum corneum treated with ethyl alcohol demonstrated a loss of structural components in the superficial cell layers of the stratum corneum. The oleic acid/ ethyl alcohol preparation transformed the highly compact cells of stratum corneum into a looser network of filaments.

Bogen KT, Keating GA, Meissner S, Vogel JS. **Initial uptake kinetics in human skin exposed to dilute aqueous trichloroethylene in vitro.** J Expo Anal Environ Epidemiol 1998;8(2):253-71.
In vitro uptake of 14C-labeled trichloroethylene (TCE) from dilute (approximately 5-ppb) aqueous solutions into human surgical skin was measured using accelerator mass spectrometry (AMS). We analyzed 105 breast-tissue samples obtained from three subjects, representing 27 separate exposure experiments conducted at approximately 20 degrees C for 0, 1, 5, 15, 30, or 60 min. The AMS data obtained positively correlate with (p approximately 0) and vary significantly nonlinearly with (p = 0.0094) exposure duration. These data are inconsistent (p approximately 0)

with predictions made for TCE by a proposed U.S. Environmental Protection Agency (USEPA) dermal-exposure model, even when uncertainties in its recommended parameter values for TCE are considered, but are consistent (p = 0.17) with a 1-compartment model for exposed skin-surface tissue governed in vitro by a maximum effective permeability of K*p = 0.28 cm h-1 (+/- 7.0%) and a first-order rate constant of k1 = 1.2 h-1 (+/- 16%). The apparent compartment depth is estimated to be approximately 40-100 microns, i.e., to comprise much or all of the epidermis. In contrast, the USEPA model implies only negligible TCE penetration beyond SC during a 1-h exposure. The K*p estimate based on the 1-compartment model fit is consistent with estimates for TCE based on in vivo studies, which supports the hypothesis that the USEPA model underpredicts short-term dermal uptake of TCE from water. It is shown that for humans, this fit also implies that normalized total uptake of TCE from water by short-term dermal contact in vivo is predicted to be fK*p, where f is approximately 80% for longer normothermic exposures and approximately 95% during a brief hot shower or bath. This study illustrates the power of AMS to facilitate analyses of contaminant biodistribution and uptake kinetics at very low environmental concentrations.

Bosman IJ, Ensing K, De Zeeuw RA. **Unusual reduction of the in vitro skin permeation of (3H)dexetimide by atropine.** Pharm Res 1998 Jan;15:145-8.
IPA COPYRIGHT: ASHP The influence of atropine on the permeation of (3H)dexetimide hydrochloride was studied in vitro in Franz diffusion cells using a donor solution of (3H)dexetimide hydrochloride with or without atropine and 3 types of membranes, fresh and frozen pig epidermal membranes and silicone rubber (Silastic) membranes. In the presence of atropine, the permeation of (3H)dexetimide hydrochloride through fresh and frozen pig epidermal membranes was decreased by about a factor of 3 and through silicone rubber was decreased by about a factor of 1.4.

Corsini E, Primavera A, Marinovich M, Galli CL. **Selective induction of cell-associated interleukin-1alpha in murine keratinocytes by chemical allergens.** Toxicology 1998;129(2-3):193-200.
Cytokines may be useful tools to discriminate between irritant and allergic contact dermatitis. In the mouse only, it has been demonstrated by other, that contact sensitizers up-regulated keratinocytes-derived interleukin-1alpha (IL-1), macrophage inflammatory protein-2 and interferon induced protein 10 mRNAs. The purpose of this study was to investigate the possibility to use in vitro IL-1 production by a murine keratinocyte cell line for preliminary screening of chemicals for their irritant and/or allergic potential. We investigated the effects of five relevant skin allergens (dinitrochlorobenzene, oxazolone, nickel sulfate, penicillin G and eugenol), two skin irritants (benzalkonium chloride, and methylsalicilate) and two compounds with no sensitizing activity (glycerol and ethanol) on IL-1 production in HEL30 cells. Twenty four hours following treatment, both IL-1 release in conditioned media and cell-associated IL-1 were measured by a specific sandwich ELISA. Under our experimental conditions, only contact sensitizers were able to increase in a dose dependent fashion cell-associated IL-1, confirming the in vivo findings. Both skin irritants and allergens induced the release of IL-1, because of the irritative properties of both chemicals, while ethanol and glycerol failed to induce changes in IL-1 production, confirming the specificity of the proposed test. Taken together, these data indicate that it may be realistic to consider potential skin allergens those chemicals which are able to increase cell-associated IL-1, to consider skin irritants those chemicals which induce only IL-1 release, and to exclude as potential allergens or irritants those chemicals which fail to induce changes in IL-1 production.

Dupuis L, Manfait M, Serpier H, Capon F, Kalis B. **Influence of ions on the hydration efficacy of urea: study using pig skin ex vivo.** Int J Cosmet Sci 1997;19(1):37-44.
IPA COPYRIGHT: ASHP The penetration of pig skin by water in oil emulsions containing both 10% urea and 10% sodium chloride or 10% magnesium sulfate and a gel containing both 10% urea and 10% sodium chloride was investigated ex vivo. The gel formulation containing urea and sodium chloride did not hydrate or penetrate pig skin. For the water in oil emulsion containing urea and sodium chloride, the moisturizing efficacy of urea but not its penetration was increased.

Feldstein MM, Raigorodskii IM, Iordanskii AL, Hadgraft J. **Modeling of percutaneous drug transport in vitro using skin-imitating Carbosil membrane.** J Controlled Release 1998 Mar 2;52:25-40.

IPA COPYRIGHT: ASHP A comparative study of the barrier function of human skin and dimethylpolysiloxane (polydimethylsiloxane)-polycarbonate block copolymer Carbosil membrane was performed in vitro using 14 drugs spanning a wide range of structures and therapeutic classes; the drug permeability coefficients across the skin and the Carbosil membrane were examined as an explicit dependence of permeant molecular weight, melting point, solubility in aqueous solution, and octyl alcohol (octanol)-water partition coefficient. Owing to heterophase and heteropolar structure, Carbosil membranes and human skin epidermis share a common solubility-diffusion mechanism of drug transport. This synthetic membrane was shown to provide a mechanistically substantiated model for percutaneous drug absorption. It was concluded that Carbosil membranes can be used both for quantitative prediction of transdermal drug delivery rate and as a skin-imitating standard membrane in the course of in vitro drug delivery kinetics evaluation.

Fentem JH, Archer G E, Balls M, Botham PA, Curren RD, Earl LK, Esdaile DJ, Holzhuetter HG, Liebsch M. **The ECVAM International Validation Study on in vitro tests for skin corrosivity. 2. Results and evaluation by the management team.**Toxicol In Vitro 1998;12(4):483-524.
BIOSIS COPYRIGHT: BIOL ABS. As a follow-up to a prevalidation study on in vitro tests for replacing the in vivo rabbit test for skin corrosivity, an international validation study was conducted during 1996 and 1997 under the auspices of ECVAM. The main objectives of the study were to: (a) identify tests capable of discriminating corrosives from non-corrosives for selected types of chemicals and/or all chemicals; and (b) determine whether these tests could identify correctly known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals. The tests evaluated were the rat skin transcutaneous electrical resistance (TER) assay, CORROSITEX, the SKIN2TM ZK1350 corrosivity test and EPISKINTM. Each test was conducted in three independent laboratories. 60 coded chemicals were tested. All of the tests evaluated showed acceptable intralaboratory and interlaboratory reproducibilities, and the TER, Skin2 and EPISKIN tests proved applicable to testing a diverse group of chemicals of different physical forms, including organic acids, organic bases, neutral organics, inorganic acids, inorganic bases, inorganic salts, electrophiles, phenols and soaps/surfactants. Two of the four tests evaluated, the TER assay and EPISKIN, met the criteria agreed by the Management Team concerning acceptable underprediction and overprediction rates. for them to be considered scientifically validated for use as replacements for the animal test for distinguishing between corrosive and non-corrosive chemicals for all of the chemical types studied (objective (a)). EPISKIN was the only test able to distinguish between known R35 (UN packing group I) and R34 (UN packing groups II & III) chemicals, for all of the chemical types included, on an acceptable number of occasions (objective (b)). The corrosive potentials of about 40% of the test chemicals could not be assessed with CORROSITEX, and the assay did not meet all of the criteria for it to be considered acceptable as a replacement test. However, CORROSITEX may be valid for testing specific classes of chemicals, such as organic bases and inorganic acids. The Skin2 assay did not meet the criteria for it to be considered scientifically validated. Thus, the validities of (i) the TER and EPISKIN assays for discriminating corrosives from non-corrosives from non- corrosives, and (ii) the EPISKIN assay for identifying correctly known R35/I and R34/II & III chemicals, have been demonstrated in this study. CORROSITEX appears to be valid when used only lid when used only with certain types of chemicals.

Goffin V, Pierard-Franchimont C, Pierard GE. **Shielded corneosurfametry and corneoxenometry: novel bioassays for the assessment of skin barrier products.** Dermatology 1998;196(4):434-7.
OBJECTIVES: One of the most frequent occupational and environmental insults to the skin is linked to chronic exposure to weak irritants. There is a need for new predictive tests assessing the efficacy of barrier creams. METHODS: Shielded variants of corneosurfametry and corneoxenometry are introduced as novel ex vivo bioassays applicable for comparing protection to surfactants and organic solvents. RESULTS: Both bioassays showed good reproducibility for each offending agent and skin-protective products. Significant differences in efficacy were indicated between the presumptive barrier products. CONCLUSIONS: Shielded corneosurfametry and corneoxenometry may be convenient bioassays to compare the protection afforded by topical products against specific offending compounds to the skin. They avoid animal testing and toxicological hazards in human testing. In addition, they are cheap, rapid and reproducible.

Goldberg AM, Maibach HI. **Dermal toxicity: alternative methods for risk assessment.** Environ Health Perspect

1998;106(Suppl 2):493-6.
Conceptually, irritant contact dermatitis (irritation) and allergic contact dermatitis (ACD) in man should provide the ideal platforms to launch in vitro toxicology into the pantheon of in vitro testing assays. In theory, irritant dermatitis has been considered by most a simple area of cutaneous biology, whereas ACD is a complex area of biology. However, both result in responses that are reasonably stereotypical and well characterized. The biology of the underlying mechanisms is becoming characterized and will thus allow development of mechanistically based in vitro assays that will be scientifically validated and thus acceptable to regulatory agencies.

Kant MV, Saini TR. **Studies on skin permeation of piroxicam.** Indian Drugs 1997 Nov;34:656-62.
IPA COPYRIGHT: ASHP The influence of some absorption enhancers on the permeation of piroxicam through human skin was studied in vitro; computer generated surface response curves were used to determine the effects of the additives. An increase in loading concentration in the donor compartment increased piroxicam permeation, indicating the first-order nature of the diffusion process. The permeation rate increased from 84 mcg/h to 165 mcg/h when the loading concentration increased from 0.5 to 2%. Dimethyl sulfoxide improved drug permeation, with a significant increase at concentrations above 20%, when the rate increased from 85 mcg/h to 229 mcg/h. With urea, permeation initially increased, but subsequently decreased at urea concentrations greater than 3%. Laurocapram decreased the drug permeation rate.

Lee KS, Kim SJ, Ryoo YW, Kim BC. **All-trans-retinoic acid down-regulates elastin promoter activity elevated by ultraviolet B irradiation in cultured skin fibroblasts.** J Dermatol Sci 1998;17(3):182-9.
Topical tretinoin therapy produces clinical improvements in the fine wrinkling of photodamaged skin, possibly by enhancement of collagen synthesis. A major biochemically and histologically detectable change in photodamaged skin is the accumulation of abnormal elastic fibers (elastotic material). However, little is known about the effects of retinoic acid and ultraviolet B (UVB) on elastin gene expression. Consequently, we examined the effects of all-trans-retinoic acid (t-RA) and UVB on elastin gene expression in cultured human skin fibroblasts in vitro. Elastin mRNA gene expression was up-regulated in response to UVB by approximately equal to 3-fold, in a dose dependent manner, between 3 and 10 mJ/cm2 doses. Similar results were obtained by chloramphenicol acetyltransferase assay, in which a maximal promoter activation more than 5.4-fold that in nonirradiated controls occurred after a single dose of 20 mJ/cm2. Also t-RA inhibited the increase in elastin mRNA level following a single exposure to UVB by approximately 16%, and the increase in promotor activity by about 65%. The inhibitory effect of t-RA on elastin induced by UVB was also demonstrated by indirect immunofluorescence studies. Taken together, t-RA down-regulated human elastin gene expression elevated by a single exposure to UVB at transcriptional and possibly protein levels. These results suggest that the anti-photoaging effect of t-RA may be related, at least in part, to down-regulation of elastin gene expression elevated by UVB.

Magnusson BM, Runn P, Karlsson K, Koskinen LO. **Terpenes and ethanol enhance the transdermal permeation of the tripeptide thyrotropin releasing hormone in human epidermis.** Int J Pharm 1997 Nov 14;157:113-21.
IPA COPYRIGHT: ASHP The effects of different penetration enhancers, including ethyl alcohol (ethanol) and the terpenes eucalyptol (cineole), carveol, and menthone, on the transdermal permeation of protirelin (thyrotropin releasing hormone), a tripeptide, in human epidermis were studied using flow through diffusion cells with an infinite dosing technique. The presence of 50% ethyl alcohol enhanced the transdermal permeation of protirelin. Mixtures of individual terpenes (3%) and ethyl alcohol (47%) further enhanced the permeation of protirelin. The major advantage of mixtures of individual terpenes and ethyl alcohol vs ethyl alcohol alone was a more rapid achievement of steady state flux.

Mempel M, Schmidt T, Weidinger S, Schnopp C, Foster T, Ring J, Abeck D. **Role of Staphylococcus aureus surface-associated proteins in the attachment to cultured HaCaT keratinocytes in a new adhesion assay.** J Invest Dermatol 1998;111(3):452-6.
Colonization of human skin with Staphylococcus aureus is a common feature in a variety of dermatologic diseases. In order to reproducibly investigate the adherence of Staphylococcus aureus to human epidermal cells, an in vitro

assay was established using the biotin/streptavidine labeling system and the HaCaT cell line. This assay was used to define the role of several Staphylococcus aureus surface proteins with regard to their function in the staphylococcal adhesion process. Our studies included the standard laboratory strain Newman as well as its genetically constructed mutants DU5873, DU5852, DU5854, and DU5886 generated by allele replacement or transposon mutagenesis, which are deficient in the elaboration of staphylococcal protein A (spa), clumping factor (clfA), coagulase (coa), and the fibronectin-binding proteins A and B (fnbA/B), respectively. In comparison with strain Newman all mutants showed remarkably reduced adherence to the HaCaT keratinocyte cell line in our assay, yielding only between 43% and 60% of the adherence capacity of strain Newman after 60 min. Bacterial adherence could be re-established by introducing the cloned wild-type genes for the surface proteins on shuttle plasmids into the chromosomally defective mutants, thus suggesting a pathogenetic role of these proteins in the attachment of Staphylococcus aureus to human keratinocytes. Bacterial adherence was additionally enhanced by alkaline pH-values that are characteristic for skin conditions with epidermal barrier dysfunction. The use of Staphylococcus aureus mutant strains, deficient in the elaboration of defined proteins, allows specific investigation of colonization and virulence factors of this dermatologic relevant microorganism.

Neubert RH, Schmalfuss U, Huschka C, Wohlrab WA. **[Recent developments in the area of dermal drug application].** Pharm Ind 1998;60(2):149-56.
IPA COPYRIGHT: ASHP A review of recent developments regarding absorption enhancers, absorption reducers/ retarders, and different vehicle systems, including liposomes, microemulsions, nanoparticles, nanocapsules, and lyotropic phases, for use in topical preparations that are intended to influence drug skin penetration is presented.

Petrali JP, Lopolito PT, Oglesby-Megee SB, Hamilton TA, Rhoads LS. **Ultrastructural and immunohistochemical characterization of an in vitro model of human epidermis: its potential for sulfur mustard study.** J Toxicol Cutan Ocul Toxicol 1997;16(3):135-44.
An in-vitro model of human skin for investigating morphological changes associated with sulfur-mustard (505602) (SM) toxicity was evaluated. The model was constructed by seeding third passage normal human keratinocytes cultured in keratinocyte growth medium (KGM) supplemented with 10% fetal bovine serum and 1.5 millimolar calcium-chloride onto Millipore Milli-Cm 10 millimeter inside diameter inserts that had been precoated with rat tail derived type-I collagen. The cells were then cultured for 7 days in the KGM after which they were examined to evaluate ultrastructural characteristics. Expression of bullous-pemphigoid-antigen (BPA) and hemidesmosomal-anchoring-filament-protein (GB3) by the cultures was evaluated by immunohistochemical techniques. The model had the epidermal structural components of human skin typically found in-vivo. For example, evidence of stratification of the epidermal cells into six to eight layers each containing cells characteristic of the stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum were seen. BPA and GB3 were immunohistochemically localized in the basal cell plasmalemma, hemidesmosomes, and subadjacent areas of the lamina lucida, the correct anatomic localizations for these proteins, as known from in-vivo studies. The authors conclude that this model should be suitable for replicative studies of SM induced skin lesions.

Rao PR, Diwan PV. **Formulation and in vitro evaluation of polymeric films of diltiazem hydrochloride and indomethacin for transdermal administration.** Drug Dev Ind Pharm 1998;24(4):327-36.
IPA COPYRIGHT: ASHP Ethylcellulose-povidone (polyvinylpyrrolidone) films containing diltiazem hydrochloride and indomethacin were prepared for transdermal drug delivery, and the effects of film composition, initial drug content, and film thickness on drug release and permeation through rat skin were studied in vitro. The drug content of the film decreased at an apparent first order rate, whereas the amount of drug released was proportional to the square root of time. Release rates of both drugs increased linearly with increasing drug content and povidone fraction in the film, but was independent of film thickness. Drug release followed a diffusion controlled model at low drug concentrations. However, a burst effect was seen initially at high drug loading levels. Skin permeation profiles showed increased flux with an increased initial drug content in the film and with the povidone concentration.

Roguet R, Cohen C, Robles C, Courtellemont P, Tolle M, Guillot JP, Duteil XP. **An interlaboratory study of the**

reproducibility and relevance of episkin, a reconstructed human epidermis, in the assessment of cosmetics irritancy. Toxicol In Vitro 1998;12(3):295-304.
BIOSIS COPYRIGHT: BIOL ABS. Reconstructed epidermal models are particularly suited to assessing the tolerance of cosmetic and dermatological products in vitro. Their production in kit form makes them available for screening both raw ingredients and finished products since a large amount of material can be tested whatever their physicochemical properties However, two conditions must first be fulfilled: they must give reproducible results and be relevant to data obtained in vivo. We tested the reproducibility of data obtained using the Episkin model (cytotoxicity evaluated by the MTT conversion and the release of one of the most active proinflammatory mediator, interleukin 1alpha(ILalpha)) on different batches and in various research laboratories. After topical application of sodium dodecyl sulfate (SDS) the overall variability of the IC50 results was 14% of the mean value. Within a given centre and a given batch, the coefficient of variation attributable to the dispersion between kits was 6% for the SDS IC50 determination and 7% for IL1alpha release measurement. The results obtained with Episkin were then compared with data from primary human skin irritancy testing (48-hr occlusion test and clinical assessment) and rabbit irritancy evaluation (Draize cutaneous test). Analysis of the results obtained with 38 cosmetic products (oils, gels, emulsions, mascaras and shaving foam, including 19 irritants) revealed good concordance with data obtained in humans. Considering the release of IL1alpha as in vitro parameter, the test sensitivity, specificity and concordance were 68, 79 and 74%, respectively.

Saravanan D. **Topical corticosteroids for skin diseases. Part 2.** Indian Drugs 1997 Feb;34:61-7.
IPA COPYRIGHT: ASHP An overview of the clinical pharmacology, pharmacokinetic properties, and clinico-pharmacological models for the assays of topically applied corticosteroids used in the treatment of skin diseases is presented, including factors affecting treatment, dosage regimens, and guidelines for topical treatment.

Sato S, Hirotani Y, Ogura N, Sasaki E, Kitagawa S. **Enhancing effect of N-dodecyl-2-pyrrolidone on the percutaneous absorption of 5-fluorouracil derivatives.** Chem Pharm Bull 1998;46(5):831-6.
The enhancing effects of N-dodecyl-2-pyrrolidone (NDP) on the percutaneous absorption of doxifluridine (DOX), 5-fluorouracil (5-FU), tegafur (TEG) and carmofur (CAR) were examined using an in vitro penetration technique and rat skin. Phosphate buffered isotonic saline (PBS), propylene glycol (PG) and PG containing 0.4M NDP (PGNDP) were applied as the donor solution. The correlation between the n-octanol/water partition coefficients and the permeability coefficients of DOX, 5-FU and TEG was investigated using both logarithmic plots. It was determined that the permeability coefficients are significantly correlated with their n-octanol/water partition coefficients on PBS. This result suggested that the non-polar stratum corneum lipid lamella in the skin might act as a rate limiting step on the skin penetration of DOX, 5-FU and TEG. The permeability coefficient of DOX, 5-FU and TEG was increased on PGNDP. The enhancing effect of NDP on the permeability coefficient was more effective at higher hydrophilic drugs, the values of the permeability coefficient had almost the same values on PGNDP and the dependency of the permeability coefficient on the n-octanol/water partition coefficient disappeared in the presence of NDP. These results indicated that the enhancing effect of NDP on the percutaneous absorption of DOX, 5-FU and TEG might be closely related to the perturbation of stratum corneum lipid lamella. Since it has been well recognized that CAR is decomposed into 5-FU in neutral and alkaline solution, the decomposition rate of CAR was measured using PBS solution and was found to be very rapid (Kd = 3.17 h-1, t1/2 = 13.1 min). The total concentrations of CAR plus 5-FU in the acceptor compartment were used to determine the permeability coefficient of CAR. The obtained value of the permeability coefficient of CAR on PG was almost the same as that of TEG on PG (CAR: 1.11 x 10(-3) cm/h, TEG: 1.24 x 10(-3) cm/h), while that of CAR on PGNDP was smaller than that of TEG on PGNDP (CAR: 6.06 x 10(-3) cm/h, TEG: 1.24 x 10(-2) cm/h). To determine the lipophilic property of CAR, the lipophilic index (log k') was measured by HPLC. The value of the lipophilic index of CAR was 92 times higher than that of TEG. These results indicated that CAR is a higher lipophilic compound, and the smaller value of the permeability coefficient of CAR compared with that of TEG on PGNDP might be caused by the strong binding of CAR to the rat skin (dermis). The dermis might act as a rate limiting step on the skin penetration of CAR, and the percutaneous absorption of CAR might be controlled by both the stratum corneum and the dermis.

Ward RK, Hubbard AW, Sulley H, Garle MJ, Clothier RH. **Human keratinocyte cultures in an in vitro approach**

for the assessment of surfactant-induced irritation. Toxicol In Vitro 1998;12(2):163-73.
BIOSIS COPYRIGHT: BIOL ABS. A specific, mechanistic, in vitro approach for the assessment of human skin irritation potential is outlined for the evaluation of surfactants and the results compared with in vivo human patch test data. The level of free available surfactant monomer and the solubilization of the corn protein zein in vitro were confirmed to be related to surfactant in vivo human skin irritation potential. In vitro cytotoxicity to monolayer keratinocyte cultures could not discriminate between the moderate human skin irritant sodium dodecyl sulfate (SDS) and the mild irritants cocamidopropylbetaine (CA) and Polysorbate 20 (P20). An in vitro stratified differentiated human epidermal equivalent (HEE) exhibited reduced cytotoxicity to the test chemicals, compared with monolayer culture responses, and was able to discriminate between the toxic potential of SDS and CA. Stimulation of interleukin-1alpha release from the A431 human keratinocyte cell line reflected in vivo erythema scores more closely than cytotoxic potential, and coincided with nitric oxide production by macrophages upon exposure to A431-conditioned medium. Combination of these mechanistic assays has allowed a profile of likely in vivo human responses to be approximated. Additional knowledge of skin penetrability and rate of recovery from toxic damage would affirm these predictions.

Wester RC, Melendres J, Sedik L, Maibach H, Riviere JE. **Percutaneous absorption of salicylic acid, theophylline, 2, 4-dimethylamine, diethyl hexyl phthalic acid, and p-aminobenzoic acid in the isolated perfused porcine skin flap compared to man in vivo.** Toxicol Appl Pharmacol 1998;151(1):159-65.
Human risk assessment for topical exposure requires percutaneous absorption data to link environmental contamination to potential systemic dose. Human absorption data are not readily available, so absorption models are used. In vitro diffusion systems are easy to use but have proved to be somewhat unreliable and are not validated to man. This study compares percutaneous absorption in the isolated perfused porcine skin flap (IPPSF) system with that in man in vivo. The study design utilized the same compounds and the same dose concentration and vehicle in both systems. Methodology for each system was that which is routinely used ineach system. The skin surface was not protected during the absorption dosing period. Percutaneous absorption values were, for man and the IPPSF system, respectively: salicylic acid (6.5 +/- 5.0%; 7.5 +/- 2.6%), theophylline (16.9 +/- 11.3%; 11.8 +/- 3.8%), 2,4-dimethylamine (1.1 +/- 0.3%; 3.8 +/- 0.6%), diethyl hexyl phthalic acid (1.8 +/- 0.5%; 3.9 +/- 2.4%), and p-aminobenzoic acid (11.5 +/- 6.3%; 5.9 +/- 3.7%) (correlation coefficient was 0.78; $p < 0.04$). The skin surface wash recovery postapplication was similar for salicylic acid in man (53.4 +/- 6.3%) and the IPPSF system (48.2 +/- 4.9%). With the other compounds the majority of surface chemical was recovered in the surface wash and skin tape strip in the IPPSF system. With man, other than salicylic acid, only a few percent applied dose was recovered with surface washing and tape stripping. Since the wash procedure was effective with pig skin, we can assume that these chemicals in man were lost to adsorption to any clothing or bedding with the volunteers. The absorption in man was not less than that in the IPPSF. Assuming the dose was lost in man, it seems plausible that whatever compound was to penetrate human skin in solvent vehicle did so in the period of time before the chemical was removed. The IPPSF system appears to be a good model for predicting percutaneous absorption relative to man. This study design should be used to validate other systems to humans in vivo. Copyright 1998 Academic Press.

Zhai H, Poblete N, Maibach HI. **Stripped skin model to predict irritation potential of topical agents in vivo in humans.** Int J Dermatol 1998;37(5):386-9.
BACKGROUND AND OBJECTIVE: The prediction of the irritation effects of products of low irritation potential remains problematic. An in vivo human model was utilized to define the irritation potential of a topical agent after partial removal of the stratum corneum by cellophane tape stripping. METHODS: The tape was applied to and removed approximately 50 times (mean, 50.0 +/- 16.7) from each test site on the volar aspect of the forearm. One site served as the stripping control, receiving tape stripping only. The other test sites received the topical agent and placebo control. Transepidermal water loss (TEWL) was measured before and daily for 5 days. The TEWL values at baseline after stripping represented the point of maximal stripping barrier disruption. The barrier disruption and irritation potential were assessed with TEWL measurements. RESULTS: The results showed that the model topical agent had no adverse effect on barrier repair, i.e. did not interfere with TEWL normalization. CONCLUSIONS: This model provides a method for the prediction, with exaggerated sensitivity, of chemical irritation and proclivity to enhance or retard water barrier repair. We believe that the model may predict the response of low irritation

materials and may be more sensitive than patch testing on normal skin, particularly for products to be used on certain areas, e.g. the face, anus, etc., or even mucous membranes. The model must receive extensive use with chemicals of varying properties in order to define its chemical relevance.

Zlotogorski A, Goldenhersh M, Shafran A. **A model for quantitative measurement of sulfur mustard skin lesions in the rabbit ear**. Toxicology 1997;120(2):105-10.
The rabbit ear was evaluated as an in-vivo model suitable for studying the pathology of and possible treatments for skin lesions produced by sulfur-mustard (505602) (SM). SM at concentrations of 0 to 500 micrograms (microg) per square centimeter (cm2) was applied to well defined 1cm2 sites on depilated skin on the ears of male albino-rabbits. The extent of edema formation was assessed from measurements of ear thickness at 12, 24, and 48 hours (hr) after application. Erythema was scored subjectively at these times using the Draize scale. Excisional biopsies were taken from the treated skin areas of some animals after 24hr for histological evaluation. A linear dose related increase in edema formation was seen in site treated with 25 to 150microg/cm2 SM at all time points. The maximum response was seen at 12hr. The edema began resolving after the 12hr evaluation point, the edematous areas decreasing by 12% at 24hr and by an additional 11% by 48hr. Subjective ratings of erythema could not be correlated with SM dose. The biopsy specimens showed significant increases in inflammatory cell infiltration, necrosis, and vesiculation as well as increases in skin thickness. The authors conclude that the rabbit ear is a useful model for examining all aspects of SM skin toxicity as well as a screening tool.

Zunic M, Bahr GM, Mudde GC, Meingassner JG, Lam C. **MDP(Lysyl)GDP, a nontoxic muramyl dipeptide derivative, inhibits cytokine production by activated macrophages and protects mice from phorbol ester- and oxazolone-induced inflammation.** J Invest Dermatol 1998;111(1):77-82.
High levels of pro-inflammatory cytokines and nitric oxide are proposed to orchestrate pathophysiologic mechanism (s) associated with various inflammatory dermatoses. This study examines whether a water soluble 3-O-[N-acetylmuramyl-L-lysyl-D-iso]-2-di-on-glycine [MDP(Lysyl)GDP], a nontoxic and nonpyrogenic derivative of muramyl dipeptide (MDP), can inhibit the in vitro production of inflammatory mediators by lipopolysaccharide- or interferon-gamma-activated macrophages, and whether such an inhibitory effect can translate into in vivo protection of mice from irritant and allergic contact dermatitis. Thioglycollate-elicited peritoneal macrophages cultured in medium alone or in medium supplemented with MDP(Lysyl)GDP (1-100 microg per ml) expressed neither mRNA transcripts for inducible nitric oxide synthase, interleukin-1beta, and tumor necrosis factor-alpha, nor cytokine proteins and nitric oxide activity. Incubation of the cells with either lipopolysaccharide or interferon-gamma for 6 h resulted in a significant induction of inducible nitric oxide synthase, interleukin-1beta, and tumor necrosis factor-alpha mRNA, and the accumulation of high levels of monokines and nitrites in cultures by 24 h. Co-incubation of the macrophages with lipopolysaccharide or interferon-gamma and MDP(Lysyl)GDP (1-100 microg per ml) resulted in a concentration-dependent suppression of the steady-state mRNA transcripts for inducible nitric oxide synthase, tumor necrosis factor-alpha, and interleukin-1beta, induced by lipopolysaccharide, but not by interferon-gamma. In mouse models of phorbol ester- and oxazolone-induced ear inflammation, topical application of MDP(Lysyl)GDP significantly suppressed ear swelling in a dose-dependent manner. Likewise, oral treatment with MDP(Lysyl)GDP at days -3, -2, and -1 before elicitation with oxazolone also significantly inhibited ear inflammation. Taken together, our findings suggest that MDP(Lysyl)GDP has the potential to be a therapeutic application in the treatment of inflammatory conditions in which overproduction of pro-inflammatory mediators are implicated to play a pathogenic role.

ECOTOXICITY

Argese E, Bettiol C, Ghirardini AV, Fasolo M, Giurin G, Ghetti PF. **Comparison of in vitro submitochondrial particle and microtox assays for determining the toxicity of organotin compounds.** Environ Toxicol Chem

1998;17(6):1005-12.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity of 14 organotin compounds was investigated by means of two short-term in vitro bioassays, the submitochondrial particle (SMP) test and the Microtox test. The first bioassay makes use of SMPs and is based on the effects of toxicants on reverse electron transport, which is induced by adenosine triphosphate and succinate at the first site level of the respiratory chain. Microtox is a well known test system that uses marine luminescent bacteria and quantifies toxicity by measuring the reduction of luminescence caused by toxic chemicals. Very good agreement was observed between the median effective concentration (EC50) values determined for organotin compounds by means of the two bioassays. Toxicity depended on both the number and kind of organic substituents bound to the tin atom. It followed the order triorganotins > diorganotinsin and phenyltin compounds exhibited the highest toxicity. Microtox and SMP EC50 values were successfully correlated with toxicity data for aquatic organisms, demonstrating the usefulness of these bioassays as prescreening or complementary tools for monitoring aquatic toxicity. Moreover, to investigate the suitability of the two assays in providing information on the mechanism of toxic action of organotins, EC50 values were correlated with various physicochemical and structural parameters of the tested compounds. The results obtained showed that these parameters are poor descriptors of organotin toxicity; in particular, the poor correlations found between EC50 values and log Kow could be ascribed to the fact that different modes of action govern the biological activity of mono-, di-, tri-, and tetraorganotin compounds, respectively.

Azuine MA, Ibrahim K, Enwerem NM, Wambebe C, Kolodziej H. **Protective role of Kigelia africana fruits against benzo(a)pyrene-induced forestomach tumorigenesis in mice and against albumin induced inflammation in rats.** Pharm Pharmacol Lett 1997;7(2-3):67-70.
IPA COPYRIGHT: ASHP The ethyl alcohol (ethanol) extract of the fruit of Kigelia africana was tested for antitumor and anti-inflammatory activity in vitro and in rats and mice. The extract showed moderate cytotoxic activity in the brine shrimp nauplii bioassay. The acute toxicity test showed an LD50 after intraperitoneal injection of 1.3 g in mice. Oral administration of the extract to mice resulted in a significant inhibition in tumor incidence and burden by 67% and 76%, respectively, in the forestomach tumorigenesis model. The extract also exhibited marked anti-inflammatory activity in rats.

Bat L, Raffaelli D. **Sediment toxicity testing: a bioassay approach using the amphipod Corophium volutator and the polychaete Arenicola marina.** J Exp Marine Biol Ecol 1998;226(2):217-39.
BIOSIS COPYRIGHT: BIOL ABS. In this study, the amphipod Corophium volutator and the polychaete Arenicola marina were evaluated as test organisms for use in sediment toxicity bioassays by adapting standard protocols developed by the US Environmental Protection Agency (EPA) and US Army Corps of Engineers (COE) (1991) and Thain et al. (1994). Although these species have begun to be used for the assessment of the toxicity of marine and estuarine sediments, the detailed ecotoxicologies of these species are not well documented. In particular, the effects of specific contaminants of known concentrations on these species in this bioassay context are not known. Here, we report experiments carried out on both species using clean intertidal sediment contaminated with copper, zinc and cadmium. The results show clear dose-dependent effects of each metal on the survival and behaviour of both species, demonstrating the bioassay has considerable potential. However, the toxicity of copper was quite different for the two species and it is recommended that several taxa should be employed in such bioassays.

Besselink HT, Denison MS, Hahn ME, Karchner SI, Vethaak AD, Koeman JH, Brouwer A. **Low inducibility of CYP1A activity by polychlorinated biphenyls (PCBs) in flounder (Platichthys flesus): characterization of the Ah receptor and the role of CYP1A inhibition.** Toxicol Sci 1998;43(2):161-71.
Several studies have reported a low inducibility of hepatic cytochrome P4501A (CYP1A) activity in European flounder (Platichthys flesus) following exposure to mixtures of polychlorinated biphenyls (PCBs). Here we report on mechanistic studies toward understanding this low CYP1A inducibility of flounder, involving molecular characterization of the Ah receptor (AhR) pathway as well as inhibition of the CYP1A catalytic activity by PCB congeners. Hepatic cytosolic AhR levels in flounder were determined using hydroxylapatite, protamine sulfate adsorption analysis, or velocity sedimentation on sucrose gradients. AhR levels in flounder (approximately 2-7 fmol/mg protein) were much lower than observed generally in rodents (approximately 50-300 fmol/mg protein). Molecular

characterization of the flounder AhR was provided by first-strand cDNA synthesis and amplification of flounder hepatic poly(A)+ RNA using RT-PCR. A 690-bp product was found, similar in size to a Fundulus AhR cDNA. The specificity of the 690-bp band was established by Southern blotting and hybridization with a degenerate AhR oligonucleotide. The deduced amino acid sequence of the flounder AhR fragment was 59-60% identical to mammalian AhR sequences. Although the AhR is present in flounder cytosol, we were unable to demonstrate detectable amounts of inducible TCDD-AhR-DRE complex in gel-retardation assays. High induction levels of CYP1A protein and associated EROD activity have been previously found in flounder following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In contrast, the induction of CYP1A catalytic activity by PCB mixtures remains unexpectedly low. Therefore, we further characterized the inhibitory potential of PCB congeners on CYP1A activity in flounder and compared this with inhibitory effects of PCB congeners on rat CYP1A activity. Analysis in vitro demonstrated that 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB, 2,2',4,4',5,5'-hexaCB, 3,3',4,4',5,5'-hexaCB, and the commercial PCB mixture Clophen A50 are potent competitive inhibitors of hepatic microsomal CYP1A catalytic activity in flounder and rat. The K(m) for ethoxyresorufin (0.095 microM) in flounder is strikingly close to Ki's found for the tested PCBs. This emphasizes the possible involvement of PCB congeners in inhibition of EROD activity in PHAH exposed fish. Finally, our data indicate that flounder CYP1A is more efficient in metabolizing ethoxyresorufin than that of rat CYP1A.

Blaise C. **Microbiotesting: an expanding field in aquatic toxicology.** Ecotoxicol Environ Saf 1998;40(1-2):115-9.
In terms of bioanalytical development and application, environmental evolution in this century can be perceived as having gone from the "dark ages" (time period preceding the 1960s and essentially characterized by nonuse of bioassays), to the "beginning of enlightenment" (use of fish bioassays during the 1960s as screening tools for effluents and specific chemicals), to the "regulatory 1970s," when newly created environment departments in developed countries began to sanction bioassays for regulatory purposes, to the "ecotoxicological 1980s," when suites of (micro) bioassays were incorporated in various hazard assessment schemes, right up to the present "microbiotesting 1990s," when an unprecedented upsurge in development and demand for cost-effective multilevel small-scale assays is manifest. This paper offers the view that microscale aquatic toxicology is a rapidly expanding field of ecotoxicology involving numerous bioanalytical techniques developed and applied at various levels of biological organization. This is demonstrated by recalling some of the major highlights that triggered the way for increased use of microbiotesting over the past decades. It is expected that the field of microbiotesting will sustain continuing growth in the future and contribute significant diagnostic power to environmental programs requiring ecotoxicology.

Cash GG. **Prediction of chemical toxicity to aquatic organisms: ECOSAR vs. Microtox assay.** Environ Toxicol Water Qual 1998;13(3):211-6.
BIOSIS COPYRIGHT: BIOL ABS. This study was designed to compare the toxicities of various chemical explosives and related compounds to aquatic organisms as predicted by a luminescent bacterium assay (Microtox assay) and by the computer program ECOSAR. Toxicities were rated "very toxic," "toxic," or "less toxic" using the same criteria as the published bacterium assay. The two methods agree for most of the 24 compounds studied, and the disagreements are about equally divided as to which method predicts the greater toxicity. Experimental toxicity data were available for 12 of the 24 compounds. With these data, ECOSAR made eight predictions that coincided with the experimental toxicity class, while the luminescent bacterium assay predicted seven. For 17 of 21 experimental LC50 values, ECOSAR predicted log LC50 within one log unit.

Costa FO, Correia AD, Costa MH. **Acute marine sediment toxicity: a potential new test with the amphipod Gammarus locusta.** Ecotoxicol Environ Saf 1998;40(1-2):81-7.
Although amphipod toxicity tests have been successfully used in the United States to assess coastal sediment toxicity, few tests have been developed with European species. The authors have been working with the amphipod Gammarus locusta, a widely spread species along European coastal areas that is particularly abundant in the Portuguese Sado estuary. This amphipod fulfills the most important requirements of a test species. It can be easily reproduced in laboratory and it is tolerant to a broad range of sediment types. A series of tests demonstrated its sensitivity to copper and gamma-hexachlorocyclohexane (lindane) in the sediment (LC50 = 6.8 mg Cu/dry kg, 0.9%

total volatile solids; LC50 = 60.5 micrograms HCH/dry kg, 2% total volatile solids) and to some heavily contaminated field sediments. After assessment of the species sensitivity to several noncontaminant variables, an experimental protocol was designed to conduct acute sediment toxicity tests that are briefly described. Proposed is a 10-day static toxicity test at 15 degrees C and 33-34/1000 salinity, with laboratory-produced juveniles and mortality as the endpoint. General assay performance is identical to the American Society for Testing and Materials (ASTM) standard for sediment toxicity tests with marine and estuarine amphipods. The results previously obtained revealed a strong potential for this amphipod to be used in toxicological testing. Considering the wide geographic distribution of this species and its amenability for culturing, it may be an alternative or complementary test for ecotoxicological studies in other European coastal systems where the existing tests cannot be applied or do not offer a definitive solution.

Cote C, Blaise C, Schroeder J, Douville M, Michaud JR. **Investigating the adequacy of selected micro-scale bioassays to predict the toxic potential of freshwater sediments through a tier process.** Water Qual Res J Can 1998;33(2):253-77.
BIOSIS COPYRIGHT: BIOL ABS. A battery of 20 bioassays was applied to assess test and endpoint performance in detecting the toxic potential of 15 freshwater sediment samples collected in the St. Lawrence River/Great Lakes systems. The bioassays included 18 micro-scale assays performed either on whole sediment, pore water or sediment organic extracts, as well as two standardized whole sediment bioassays undertaken with macroinvertebrates (Chironomus riparius and Hyalella azteca). Physical and chemical parameters of selected sediments were also analyzed to aid in the interpretation of results. Several qualitative and quantitative criteria were established for evaluating the reliability and usefulness of the micro-scale bioassays in predicting sediment toxicity based on a two-tier system. In Tier 1, micro-scale testing results were compared for general concordance with those of the benthic bioassays, benthic community structure index and with sediment contaminant levels. Micro-assays which complied with Tier 1 standards were further evaluated via a Tier 2 process consisting of selected (quantitative and qualitative) scientific and practical criteria. Of the 20 bioassays investigated the two benthic assays and seven micro-scale assays successfully passed the double-tiered assessment. Micro-scale tests whose results most closely matched those of the benthic tests for appraisal of whole sediment toxicity, sediment contamination level and benthic community structure index included three bacterial assays (ATP assay, a microplate adaptation of the Microtox assay, SOS Chromotest), one algal assay (algal solid-phase test) and three micro-invertebrate assays (Thamnotoxkit F, Daphtoxkit F and Hydra assay). Two selected batteries, composed of the seven micro-scale assays and benthic invertebrate bioassays, are proposed for cost-effective appraisal of freshwater sediment toxicity.

Davies IM, Gillibrand PA, McHenery JG, Rae GH. **Environmental risk of ivermectin to sediment dwelling organisms.** Aquaculture 1998;163(1-2):29-46.
BIOSIS COPYRIGHT: BIOL ABS. Ivermectin (22,23-dihydroavermectin B1) has been proposed as a feed additive to alleviate sea lice infestation of farmed salmon. The present study investigated the acute toxicity of ivermectin in sediment to two sediment-dwelling organisms, the amphipod, Corophium volutator and the starfish, Asterias rubens. The degradation of ivermectin in sediment was studied over a three-month period, by chemical measurements and observation of changes in toxicity of the contaminated sediment with time to C. volutator in whole sediment bioassays. The 10 day LC50s of ivermectin to C. volutator and A. rubens were 0.18 mg kg-1 and 23.6 mg kg-1, respectively. The estimated NOECs of ivermectin for mortality to C. volutator and A. rubens were 0.05 mg kg-1 and 5 mg kg-1, respectively. The lowest observed concentration which had a sublethal effect (LOEC) on the ability of A. rubens to turn themselves over after the 10-day test was 20 mg kg-1. Measured concentrations of ivermectin in the sediment, and the decrease in toxicity, indicated that the half-life of ivermectin in marine sediments is greater than 100 days. An initial assessment of the potential risk to the marine environment from sediment-associated ivermectin indicated that there may be significant risk to infaunal polychaetes in sediment immediately below and around the fish cages.

Densmore CL, Smith SA, Holladay SD. **In vitro effects of the extracellular protein of Renibacterium salmoninarum on phagocyte function in brook trout (Salvelinus fontinalis).** Vet Immunol Immunopathol 1998;62(4):349-57.

Renibacterium salmoninarum is a facultative intracellular pathogen often found in host phagocytes where it appears to successfully avoid the host fish's immunological defenses. The objective of this investigation was to determine if soluble extracellular protein (ECP) produced by R. salmoninarum may contribute to the immunomodulation in bacterial kidney disease (BKD) via inhibition of phagocyte respiratory burst and/or phagocytosis mechanisms. Splenic cells from adult brook trout (Salvelinus fontinalis) were incubated with two different concentrations of ECP (0.1 mg/ml and 1.0 mg/ml) and viable R. salmoninarum. Splenic cell cultures were evaluated for respiratory burst activity via flow cytometry with the dichlorofluorescin diacetate (DCF-DA) assay and for phagocytosis via light microscopic assessment of microsphere engulfment. Respiratory burst activity was significantly inhibited in all treatment groups as compared to untreated fish, while no differences were noted in phagocytic activity.

Desbrow C, Rutledge EJ, Brighty GC, Sumpter JP, Waldock M. **Identification of estrogenic chemicals in STW effluent: 1. Chemical fractionation and in vitro biological screening.** Environ Sci Technol 1998;32(11):1549-58.
BIOSIS COPYRIGHT: BIOL ABS. A fractionation system, combined with an in vitro assay for detecting estrogenic activity, was developed in order to isolate and identify the major estrogenic chemicals present in seven sewage-treatment works (STW) effluents, receiving primarily domestic effluent, discharging into British rivers. Three sterols were isolated from estrogenic fractions of sewage extracts; these were the natural hormones 17beta-estradiol and estrone and the synthetic hormone 17alpha-ethynylestradiol. 17beta-Estradio estrone were present in all the effluents at measured concentrations ranging from 1 ng/L to almost 50 and 80ng/L, respectively. The concentration of 17alpha-ethynylestradiol was generally below the limit of detection but was positively identified in three of the effluent samples at concentrations ranging from 0.2 to 7.0 ng/L. These data suggest that natural and synthetic hormones may be responsible for the observed induction of vitellogenin synthesis in male fish placed downstream of effluent discharges from STWs that receive mainly domestic inputs.

Diaz-Mendez FM, Rodriguez-Ariza A, Usero-Garcia J, Pueyo C, Lopez-Barea J. **Mutagenic activation of aromatic amines by molluscs as a biomarker of marine pollution.** Environ Mol Mutagen 1998;31(3):282-91.
BIOSIS COPYRIGHT: BIOL ABS. Mutagenic activation of arylamines by mollusc S9 fractions was evaluated as a biomarker for marine pollution. Two bivalve species were used as bioindicators, the common mussel (Mytilus edulis) and the striped venus (Chamelea gallina). A strain of Salmonellatyphimurium overproducing O-acetyltransferase was used as indicator of mutagenicity. Mussels from an area of the North Atlantic Spanish zone that was exposed to an accidental crude oil spill were compared to bivalves from a reference area. C. gallina samples were from low polluted and highly polluted areas of the South Atlantic Spanish littoral. The promutagen 2-aminoanthracene (2-AA) was activated to mutagenic derivative(s) by S9 fractions from both C. gallina and M. edulis. Animals from contaminated sites showed higher arylamine activation capabilities than reference animals. This was further correlated with the mutagenic activities of corresponding cyclopentane-dichloromethane animal extracts. 2-AA activation by mollusc 59 was potentiated by alpha-naphthoflavone (ANF), known to inhibit PAH-inducible CYP1A cytochromes from vertebrates, but inhibited by methimazole (MZ), a substrate of the flavin monooxygenase (FMO) system. 2-AA-activating enzymes were mainly cytosolic; this localization clearly suggests that such activity could be attributed to soluble enzymes, different from the CYP1A or FMO systems. In conclusion, mutagenic activation of arylamines by mollusc 59, using as indicator a strain of Salmonella typhimurium that overproduces O-acetyltransferase, could be a reliable biomarker for marine pollution.

Dorn PB, Vipond TE, Salanitro JP, Wisniewski HL. **Assessment of the acute toxicity of crude oils in soils using earthworms, Microtox and plant.** Chemosphere 1998;37(5):845-60.
BIOSIS COPYRIGHT: BIOL ABS. The assessment of soil quality resulting from a chemical or oil spill and/or remediation effort may be obtained by evaluating the toxicity to soil organisms. To enhance our understanding of the soil quality resulting from laboratory and field oil spill remediation, we have assessed three soil toxicity test methods. Heavy, medium and light crude oils (API gravity 16-18, 30 and 53) were spiked into two soils in the laboratory. The earthworm (Eisenia foetida) 14-d lethality assay, the modified Microbics Microtox Solid Phase assay, and the 14-d plant seed germination and growth assays were tested with combinations of crude oils and soils. Earthworms were 1.4 to 14 times more sensitive than Microtox and 1.3 to >77 times more sensitive than plants to the oily soils. Light oil in the silty low organic carbon soil was generally the most toxic, while heavy oil in

the sandy high organic carbon soil was least toxic. The bioassay techniques were demonstrated to be sensitive indicators of soil quality and may be used to evaluate the quality of remediated oily soils.

Eller-Jessen K, Crivello JF. **Subcutaneous NaAs3+ exposure increases metallothionein mRNA and protein expression in juvenile winter flounder.** Aquatic Toxicol 1998;42(4):301-20.
BIOSIS COPYRIGHT: BIOL ABS. We investigated the effect of NaAs3+ exposure on liver metallothionein (MT) mRNA and protein expression in juvenile winter flounder (Pleuronectes americanus). Sexually immature flounder (age 18 months) were subcutaneously implanted with silastic tubing containing 0 (vector control), 1. 5, 3.0, 6.0 and 16.0 mumol g-1 fish wt. NaAs3+. Liver MT mRNA and protein were significantly elevated within 24 h of exposure at the 1.5 mumol g-1 dose before there was significant liver As accumulation or mortality. All four NaAs3+ treatments caused a significant rise in MT mRNA and protein and the mRNA demonstrated the clearest dose response and greatest fold change (19 fold at 16.0 mumol g-1 dose). MT protein reached its maximum (1.8 times the control) at the 1.5 mumol g-1 dose. This maximum was maintained across all four treatments and decreased slightly at the 16.0 mumol g-1 dose where there was 40% mortality. The level of liver As content among fish with maximal MT expression was similar to that of fish which recently succumbed to NaAs3+ toxicity. This links saturation of the MT response with mortality and provides a framework for assessing the significance of MT measurements in feral flounder. The experiment was repeated (minus the 16.0 mumol g-1 dose) in a smaller (and younger) group of fish (12 months old) which expressed a two-fold lower liver Zn and MT protein content. This relatively low level of pre-existing MT theoretically widened the MT response window such that the MT maximum was not attained until the 6.0 mumol g-1 dose. These fish also did not show a significant rise in either MT mRNA or protein at the sublethal dose (1.5 mumol g-1). This lack of change appeared to reflect the higher level of baseline liver As which also did not increase at the 1.5 mumol g-1 dose. Reevaluation of the earlier experiment revealed a 1.5 fold increase in liver As content at the 1.5 mumol g-1 dose that was not significant due to high variance. This suggests that MT is a better biomarker of NaAs3 + exposure because there is less variance associated with either MT mRNA or protein measurements. These results lend merit to MTOs usefulness as a biomarker of metal exposure and provides the foundation for linking NaAs3+ exposure with MT expression and possibly metal-induced carcinogenesis in fish.

Espigares M, Crovetto G, Galvez R. **In vitro evaluation of the toxicity of several dithiocarbamates using an Escherichia coli growth inhibition bioassay and the acetylcholinesterase inhibition test.** Environ Toxicol Water Qual 1998;13(2):165-75.
BIOSIS COPYRIGHT: BIOL ABS. The industrial and sanitary use of dithiocarbamates (DTCs) is on the rise, and the synthesis of new derivatives has increased the field of their application. Both positive and negative pharmacological and toxicological effects have been documented for DTCs. The development and application of new DTCs must be accompanied by the study of their toxicity, beginning with the performance of simple and rapid biological screening tests. The objective of our study was to apply the IGEC biotest (inhibition of growth of Escherichia coli) and the acetylcholinesterase (AChE) inhibition test to a group of newly synthesized DTCs with possible applications in the area of public health. The substances studied were (-)-ephedrine-DTC, (+)-pseudoephedrine-DTC, N-ethylbenzyl-DTC, diethyl-DTC, and dimethyl-DTC, and the corresponding amines used in their synthesis: (-)-ephedrine, (+)-pseudoephedrine, N-ethylbenzylamine, diethylamine, and dimethylamine. The parameters for the minimal effective concentration tested (MEC), median effective concentration (EC50), and no observed effect concentration (NOEC) of each chemical were determined using the IGEC biotest. The AChE inhibition test was carried out for all the DTCs as well. Of the synthesized DTCs, (+)-pseudoephedrine-DTC showed the lowest toxicity (NOEC = 30 mug mL-1 and EC50 = 301 mug mL-1) and N-ethylbenzyl-DTC showed the highest toxicity (EC50 = 26 mug mL-1). Although (-)-ephedrine-DTC exhibited a minimal inhibitory effect on AChE, the results obtained indicate a generalized absence of AChE inhibition for the DTCs.

Fairey R, Roberts C, Jacobi M, Lamerdin S, Clark R, Downing J, Long E, Hunt J, Anderson B, Newman J, et al. **Assessment of sediment toxicity and chemical concentrations in the San Diego Bay region, California, USA.** Environ Toxicol Chem 1998;17(8):1570-81.
BIOSIS COPYRIGHT: BIOL ABS. Sediment quality within San Diego Bay, Mission Bay, and the Tijuana River Estuary of California was investigated as part of an ongoing statewide monitoring effort (Bay Protection and Toxic

Cleanup Program). Study objectives were to determine the incidence, spatial patterns, and spatial extent of toxicity in sediments and porewater; the concentration and distribution of potentially toxic anthropogenic chemicals; and the relationships between toxicity and chemical concentrations. Rhepoxynius abronius survival bioassays, grain size, and total organic carbon analyses were performed on 350 sediment samples. Strongylocentrotus purpuratus development bioassays were performed on 164 pore-water samples. Toxicity was demonstrated throughout the San Diego Bay region, with increased incidence and concordance occurring in areas of industrial and shipping activity. Trace metal and trace synthetic organic analyses were performed on 229 samples. Copper, zinc, mercury, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and chlordane were found to exceed ERM (effects range median) or PEL (probable effects level) sediment quality guidelines and were considered the six major chemicals or chemical groups of concern. Statistical analysis of the relationships between amphipod toxicity, bulk phase sediment chemistry, and physical parameters demonstrated few significant linear relationships. Significant differences in chemical levels were found between toxic and nontoxic responses using multivariate and univariate statistics. Potential sources of anthropogenic chemicals were discussed.

Ferdidandy-Van Vlerken M. **Chances for biological techniques in sediment remediation.** Water Sci Technol 1998;37(6-7):345-53.
BIOSIS COPYRIGHT: BIOL ABS. Biological techniques can be applied in remediation of sediments contaminated with organic pollutants, such as mineral oil, PAH, PCB and chlorobenzenes. Within the period of 1989-1997 several techniques were developed from laboratory to full-scale. The chances for bioremediation lay in the relatively small environmental impact and the low costs of the techniques. Little energy is required, no emissions to soil or air occur and the natural structure of the sediments is not destroyed. The costs vary between 45 (landfarming) and 70 (reactors) Dutch Guilders per ton dry weight. The quality of the product to meet the legal standards for re-use was achieved for some, but not all, of the sediments. However, bioassays and leaching tests showed that bioremediation strongly reduces the ecotoxicity and dispersion risks of the material. Further research of the actual risks, accompanied by policy development of the standards for re-use, might increase the number of different types of sediments which can be successfully remediated by biotechnology.

Ferrero M, Castano A, Gonzalez A, Sanz F, Becerril C. **Characterization of RTG-2 fish cell line by random amplified polymorphic DNA.** Ecotoxicol Environ Saf 1998;40(1-2):56-64.
The increasing presence of genotoxic chemicals in the aquatic environment has led to the development of both in vivo and in vitro assays for target species. The fish population represents an important level of aquatic ecosystems that can be threatened by increased environmental pollution. The authors have studied the DNA pattern of the RTG-2 fish cell line, a fibroblast-like cell line, derived from rainbow trout (Oncorhynchus mikyss), to use this cell line as an in vitro system to study genotoxicity by means of random amplified polymorphic DNA primers (RAPDs). A constant pattern in the DNA band is essential when an organism or cell line is used to detect DNA alterations produced by genotoxic environmental chemicals. DNA fingerprints with RAPDs were obtained for RTG-2 by testing 26 single and 70 pairwise combinations of primers. Different methods of DNA extraction (chelating resin, salting out, and phenolization), the influence of spectrometric measures at 320 nm in the 260/280 quotient to quantify DNA extracts, genomic DNA and primer concentrations, annealing temperatures, and cell line passage were studied in the cell line characterization. RAPD products were identified by agarose gel electrophoresis. The good results obtained should allow the use of this system as a possible tool for detection of the genotoxicity of aquatic pollutants.

Fischer U, Ototake M, Nakanishi T. **In vitro cell-mediated cytotoxicity against allogeneic erythrocytes in ginbuna crucian carp and goldfish using a non-radioactive assay.** Dev Comp Immunol 1998;22(2):195-206.
Cell-mediated cytotoxicity of clonal ginbuna crucian carp leukocytes against allogeneic erythrocytes is described using a sensitive non-radioactive in vitro assay. Hemoglobin released from target erythrocytes after cell-mediated erythrolysis was detected by tetramethylbenzidine (TMB). TMB assay showed clear correlation with a 51Cr-release assay and even exhibited higher cytotoxicity. The use of erythrocytes as target cells has several advantages over a conventional 51Cr-release assay. Erythrocytes do not have cytotoxic activity, are relatively homogeneous, are available in large numbers and erythrocyte donors need not be killed. Leukocytes from fish sensitized by erythrocyte injection or scale grafting efficiently lysed allogeneic erythrocytes, but did not kill isogeneic or

autologous erythrocytes. In contrast, leukocytes from unsensitized fish did not lyse allogeneic erythrocytes and repeated sensitizations by allogeneic grafts were necessary to induce cytotoxic cells. Effector cells isolated from peripheral blood showed a higher cytotoxic effect toward allogeneic target cells than effector cells isolated from kidney. These studies support the hypothesis that fish are capable of a genetically restricted specific cell-mediated cytotoxicity.

Gerritsen A, Van Der Hoeven N, Pielaat A. **The acute toxicity of selected alkylphenols to young and adult Daphnia magna.** Ecotoxicol Environ Saf 1998;39(3):227-32.
Differences in sensitivity toward toxicants between young and adult individuals in a population are assumed to be primarily associated with their difference in body size. This assumption plays a key role in the modeling of effects of variable concentrations of toxicants on nonhomogeneous populations. The hazard-based no-effect-concentrations (NECs), killing rates, and elimination rates, estimated from the survival data of a series of acute toxicity tests with young and adults of Daphnia magna and six alkylphenols, were used to evaluated this assumption. The results lead to the conclusion that young and adult D. magna were equally sensitive in terms of NEC and killing rate and that the observed differences in elimination rates could be explained on the basis of a difference in body size. Furthermore, it was found that elimination rates estimated on the basis of the survival data were consistently smaller than those expected on the basis of a QSAR for Daphnia pulex, a comparable species. This discrepancy was likely due to a decreased uptake and elimination during a period of immobilization prior to death. Since it is unknown to what extent immobilized individuals are able to recover from short-term exposures, the observed deviation clearly identifies a complicating factor in the modeling of effects of variable concentrations of toxicants.

Goicolea A, Barrio RJ, Gomez de Balugera Z, Gorostiza I, San Vicente A, Diaz AI. **Study of the toxicity in industrial soils by the bioluminescence assay.** J Environ Sci Health A 1998;33(5):863-75.
BIOSIS COPYRIGHT: BIOL ABS. A survey of the toxicity of soils from the several industrial zones of the Spanish Basque Country was undertaken in order to identify the relationship between chemical contamination and toxicity. The measured effect in the solid and liquid-phase Microtox toxicity test was correlated with the chemical parameters to determine the origin of the toxicity effect. Results indicate the higher sensitivity of solid-phase bioassay. In a comparative study with the liquid-phase assay it was found that due to different solubility of each contaminant in water the test on the extracts represents only a part of multiple contamination. Moreover water elutriation could underestimate the types and concentrations of organic contaminants present.

Hazel JR, McKinley SJ, Gerrits MF. **Thermal acclimation of phase behavior in plasma membrane lipids of rainbow trout hepatocytes.** Am J Physiol 1998;275(3 Pt 2):861-9.
The fluorescent probes laurdan (6-dodecanoyl-2-dimethylaminonapthalene) and N-[7-nitrobenz-2-oxa-1, 3-diazol-4-yl] dipalmitoyl-L-alpha-phosphatidylethanolamine (NBD-PE) in addition to Fourier transform infrared spectroscopy (FTIR) were employed to measure the phase behavior and physical properties of hepatocyte plasma membranes isolated from the livers of thermally acclimated (5 and 20 degreesC) rainbow trout (Oncorhynchus mykiss). The primary objective was to determine the extent to which the phase behavior of membrane lipids is conserved at different growth temperatures. Arrhenius plots of laurdan-generalized polarization revealed a single discontinuity believed to reflect either the onset of the gel-fluid phase transition or the formation of gel phase microdomains, and this discontinuity occurred at significantly higher temperatures in membranes of 20 degrees C (13.2 +/- 0.7 degrees C)- than 5 degrees C (7.2 +/- 0.1 degrees C)-acclimated trout. Similarly, acclimation from 5 to 20 degrees C increased both the onset temperature (from 2.0 +/- 0.3 to 7.2 +/- 0.6 degrees C) and the thermal range (from 10.9 +/- 0.5 to 16.0 +/- 1.0) of the gel-fluid transition as assessed by FTIR. The gel-fluid transition midpoint (approximately -2 degrees C) and completion temperatures (-9 degrees C) were unchanged by thermal acclimation. The anisotropy of NBD-PE fluorescence displayed a distinct minimum in membranes of both warm- and cold-acclimated trout (reflecting alterations in lipid packing that in pure lipid membranes ultimately lead to the formation of nonlamellar phases) in the range of 56-58 degrees C; only membranes of 5 degrees C-acclimated trout displayed an additional minimum at significantly lower temperatures (24.5 +/- 1.7 degrees C). Collectively, these data suggest that the regulation of both the temperature at which gel phase lipids begin to form in response to cooling as well as the propensity of membrane lipids to form nonlamellar phases at higher temperatures may be key features of

membrane organization subject to adaptive regulation.

Jak RG, Ceulemans M, Scholten MC, Van Straalen N. **Effects of tributyltin on a coastal North Sea plankton community in enclosures.** Environ Toxicol Chem 1998;17(9):1840-7.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity of tributyltin (TBT) to a neritic North Sea plankton community was studied in experiments using outdoor enclosures with a volume of 1.2 m3. Nominal TBT concentrations of 0, 0.056, 0.1, 0.18, 0.32, and 0.56 mug/L were tested in duplicate for 28 d. A first-order decrease of TBT in the water column was observed for the highest nominal concentration, with a rate constant of 0.12 per day (t1/2 of 5.7 d). Median effective concentration (EC50) values were determined for the dominant zooplankton species, Temora longicornis (Copepoda). The EC50 values were lower when calculated for biomass than when calculated for density and ranged between 0.15 and 0.32 mug/L, depending on exposure time. At high TBT concentrations, enhanced pH levels and oxygen concentrations were observed, indicating high algal production levels, which would result from reduced grazing by zooplankton. Depending on exposure time, EC50 values for the indirect effect of TBT on pH varied between 0.10 and 0.22 mug/L, indicating that the indirect effects of TBT are at least as sensitive as the direct effects. The study showed that testing chemicals in outdoor enclosures can provide data complementary to that obtained from laboratory toxicity tests.

Kaiser KL. **Correlations of Vibrio fischeri bacteria test data with bioassay data for other organisms.** Environ Health Perspect 1998;106(Suppl 2):583-91.
Linear relationships of the median lethal concentrations of several hundreds of chemicals for a variety of organisms with Vibrio fischeri median effective concentrations are investigated. Significant correlations can be developed for many aquatic species including the fishes fathead minnow, bluegill, catfish, goldfish, goldorfe, guppy, killifish, rainbow trout, sheepshead minnow, and zebrafish; the water flea Daphnia sp.; such crustaceans as Artemia sp. and Crangon sp.; the ciliate Tetrahymena pyriformis; and algae, such as Chlorella sp. These interspecies relationships can be used to estimate order-of-magnitude type toxic effects of many substances for these aquatic organisms. Highly significant relationships can be obtained when selecting compounds on a chemical basis, such as alcohols, ketones, aromatics, etc., which allow the calculation of the compounds' toxicities to the corresponding aquatic species with increased accuracy and confidence. Analogous correlations with mammalian (rat and mouse) oral, intraperitoneal, and intravenous median lethal dose (LD50) data are much weaker than those for most aquatic species. However, there are significant differences between these three routes of administration and the intravenous LD50 data show the best relationship with the Vibrio data.

Karcher W. **Recent trends and developments in the EU in the environmental control and management of chemicals.** Ecotoxicol Environ Saf 1998;40(1-2):97-102.
In the further development and implementation of European legislation on chemicals and related products the following aspects and trends can be identified: (1) refinement and adaptation of existing legislation and guidelines to technical progress (including extension of testing and risk assessment strategies), (2) preparation and implementation of new directives and regulations, (3) increasing application and further development of screening methods and model approaches, and (4) global harmonization of hazard and risk assessment criteria and procedures in the frame of the International Forum on Chemical Safety (chapter 19 of Agenda 21). The increasing use of evaluation and screening models can be illustrated by the further development and application of specific models such as EUSES and EASE to predict effect and exposure data. On the effects assessment side, models are available or under development to predict the phototoxicity and potential endocrine disruptors (estrogen/androgen binding affinity) of industrial chemicals. Finally, the emphasis that is given worldwide to the global harmonization procedures initiated under chapter 19 of Agenda 21, agreed at the UNCED conference for the environment and sustainable development in 1992, cannot fail to influence further developments in Europe.

Karman CC, Reerink HG. **Dynamic assessment of the ecological risk of the discharge of produced water from oil and gas production platforms.** J Hazardous Mater 1998;61(1-3):43-51.
BIOSIS COPYRIGHT: BIOL ABS. Since 1991 the North Sea countries (UK, Netherlands, Norway and Denmark) have put a lot of effort in the development of a decision support system for the legislation of the use and discharge

of offshore exploration, drilling and production chemicals. The heart of this so-called 'harmonised mandatory control system' is the 'chemical hazard assessment and risk management' (CHARM) model. This model enables the ranking of chemicals on the basis of their intrinsic properties, using a realistic worst-case scenario. To meet the prerequisites of the model (simple and transparent calculation rules), the CHARM model uses a fixed dilution factor, assuming equal and constant dispersion of chemicals around the platform. In reality, however, the chemical follows a three-dimensional dispersion pattern which will change over time. To be able to use the principles of the CHARM model in such a dynamic situation for risk management, a new model has been developed by TNO in cooperation with Dutch Oil (NAM). This model gives a probabilistic estimation of the ecological risk of produced water, based upon a realistic calculation of the fate of components of produced water after discharge from the platform. Spatial and temporal variation in the concentration of chemicals is summarised in frequency distributions. The ecological risk is calculated for aquatic life, benthic life and the food chain. The model aims to support the selection of cost-effective mitigating measures for risk reduction.

Koistinen J, Soimasuo M, Tukia K, Oikari A, Blankenship A, Giesy JP. **Induction of EROD activity in Hepa-1 mouse hepatoma cells and estrogenicity in MCF-7 human breast cancer cells by extracts of pulp mill effluents, sludge, and sediment exposed to effluents.** Environ Toxicol Chem 1998;17(8):1499-507.
BIOSIS COPYRIGHT: BIOL ABS. Extracts of effluents and sludges from the primary and secondary clarifiers of an activated sludge treatment plant at a Finnish bleached kraft pulp and paper mill were analyzed in two cell bioassays. Total dioxin-like activities were determined by measuring the induction of ethoxyresorufin-O-deethylase (EROD) activity in Hepa-1 mouse hepatoma cells. Estrogenicity was studied by measuring luciferase activity in MCF-7 ERE-luc, which are MCF-7 human breast cancer cells stably transfected with an estrogen-responsive element linked to a luciferase promoter. Sediments collected near the pulp mill and from other sites in Lake Saimaa as well as fillets of whitefish exposed to effluents were examined and EROD activity was determined for complex mixtures of compounds extractable with dichloromethane (DCM) from the pulp mill effluent or compounds collected by semipermeable membrane devices (SPMDs) from the same effluent. Extracts of effluents, particulates, SPMDs, and sludges all caused measurable EROD induction. Because the induction potencies of the total DCM extract and the fraction of neutral compounds were similar, it was concluded that most of the EROD induction in pulp mill effluents was due to neutral compounds. Sediment collected from the vicinity of the pulp mill had greater EROD-inducing potency compared to that of the control sites from the same lake. Induction of EROD potencies of muscle extracts of whitefish exposed to diluted effluents were not significantly greater than those of control fish exposed to dilution water only. All extracts contained some estrogen agonist activity when tested in the MCF-7 bioassay.

Lagriffoul A, Mocquot B, Mench M, Vangronsveld J. **Cadmium toxicity effects of growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (Zea mays L.).** Plant Soil 1998;200(2):241-50.
BIOSIS COPYRIGHT: BIOL ABS. Plants were cultivated in a nutrient solution containing increasing cadmium concentrations (i.e. 0.001-25 muM), under strictly controlled growth conditions. Changes in both growth parameters and enzyme activities, directly or indirectly related to the cellular free radical scavenging systems, were studied in roots and leaves of 14-day-old maize plants (Zea mays L., cv. Volga) as a result of Cd uptake. A decrease in both shoot length and leaf dry biomass was found to be significant only when growing on 25 muM Cd, whereas concentrations of chlorophyll pigments in the 4th leaf decreased from 1.7 muM Cd on. Changes in enzyme activities occurred at lower Cd concentrations in solution leading to lower threshold values for Cd contents in plants than those observed for growth parameters. Peroxidase (POD; E.C. 1.11.1.7) activity increased in the 3rd and 4th leaf, but not in roots. In contrast, glucose-6-phosphate dehydrogenase (G6PDH; E.C. 1. 1. 1.49), isocitrate dehydrogenase (ICDH; E.C. 1.1.1.42) and malic enzyme (ME; E.C. 1.1.1.40) activities decreased in the 3rd leaf. According to the relationship between the POD activity and the Cd content, a toxic critical value was set at 3 mg Cd per kg dry matter in the 3rd leaf and 5 mg Cd per kg dry matter in the 4th. Ar ionic POD were determined both in root and leaf protein extracts; however, no changes in the isoperoxidase pattern were detected in case of Cd toxicity. Results show that in contrast with growth parameters, the measurement of enzyme activities may be included as early biomarkers in a plant bioassay to assess the phytotoxicity of Cd-contaminated soils on maize plants.

Lopez-Barea J, Pueyo C. **Mutagen content and metabolic activation of promutagens by molluscs as biomarkers of marine pollution.** Mutat Res 1998;399(1):3-15.
Organisms combat pollutants by inducing biotransformation pathways, which can be used for biomonitoring. Several parameters--biomarkers--change in stressed organisms or populations at different organisation levels. Molecular or cellular biomarkers are early-warning indicators of pollution. Xenobiotics are first biotransformed by phase I enzymes and then conjugated with endogenous metabolites by phase II enzymes. Many organic xenobiotics are initially biotransformed by cytochrome P4501A1; in mammals, it is induced by pollutants via Ah receptor, although in marine invertebrates, its inducibility is much more equivocal. Metallothioneins are small Cys-rich proteins which bind transition metals; they detoxicate pollutant metals and are clearly induced in metal-exposed marine invertebrates. Some pollutants are genotoxins or can be converted into them. Determination of mutagens in bivalve molluscs following extraction with solvents and assay of mutagenicity with bacterial tests is a useful biomarker for marine pollution. While some pollutants are directly mutagenic, others are only mutagenic after they are activated to mutagenic derivatives by monooxygenases or conjugative enzymes. Many of these catalysts are induced by xenobiotics; thus, increased activation of known promutagens can be used as biomarker of environmental pollution. Bioactivation of promutagens requires the simultaneous action of different pathways, thus, reproducing more closely the in vivo situation than the specific assay of individual biotransforming enzymes. Study of molluscs with different pollution levels indicates that polluted animals have higher capacity to activate 2-aminoanthracene and contain more apolar mutagens than those from reference areas.

Low KW, Sin YM. **Effects of mercuric chloride and sodium selenite on some immune responses of blue gourami, Trichogaster trichopterus (Pallus).** Sci Total Environ 1998;214:153-64.
The immunotoxicological effects of mercuric chloride and sodium selenite on blue gourami were studied. Some immune responses ranging from non-specific to specific were investigated. These include tissue lysozyme activity, kidney lymphocyte proliferation and plasma agglutinating antibody titre against bacteria. After 2 weeks of chronic exposure, 0.09 mg/l of Hg2+ alone induced a significant increase of kidney lysozyme activity of 4196.3 +/- 1171.0 U/g, but it decreased to 1577.4 +/- 902.4 U/g when exposed simultaneously to equiconcentration of selenium. Plasma lysozyme activity was also increased by co-administration of Hg2+ and SeO3(2-). The level of plasma agglutinating antibody against Aeromonas hydrophila L37 was lowered in the chemical-treated fish. This indicates that the fish immunity was impaired by action of mercury and selenium. However, the in vitro lymphocyte proliferation test shows that mercury concentration lower than 0.045 mg/l Hg2+ enhanced the mitotic rate of kidney lymphocytes by approximately 30%. A high concentration of mercury caused irreversible damaging effects on con A-induced lymphoblastogenesis. In contrast, the inhibitory effect of low concentrations of mercury could be removed by washing. On the other hand, selenium showed a suppressive effect on the lymphocyte proliferation even at 0.5 mg/l.

Marwood TM, Knoke K, Yau K, Lee H, Trevors JT, Suchorski-Tremblay A, Flemming CA, Hodge V, Liu DL, Seech AG. **Comparison of toxicity detected by five bioassays during bioremediation of diesel fuel-spiked soils.** Environ Toxicol Water Qual 1998;13(2):117-26.
BIOSIS COPYRIGHT: BIOL ABS. Biodegradation of petroleum contaminants is an effective and generally inexpensive approach for reducing their concentrations in soils. However, little information is available on the toxicological status of contaminated soils and the fate of target hydrocarbons following bioremediation. Four texturally distinct soils were contaminated with diesel fuel and bioremediated in microcosms at 22C, with moisture contents of 85% of soil water holding capacity, and nitrogen (N) and/or phosphorus (P) nutrient amendments. The progress of bioremediation was monitored using chemical and toxicological analyses. Soil toxicity was measured using five short-term bioassays: seed germination, red blood cell hemolysis, solid-phase Microtox, SOS-chromotest, and Toxi-chromotest. Reductions in target compound concentration were not always predictive of reductions in soil toxicity. Conflicting trends were indicated by the toxicity test results. For example, total petroleum hydrocarbon analysis revealed decreased hydrocarbon concentrations in all four soils following bioremediation but seed germination and seedling emergence data indicated increased soil toxicity. In contrast, the Microtox test data indicated decreased toxicity in two of the four soils. These results suggest that measurements of target contaminant concentrations should be complemented with several different soil toxicity bioassays, particularly when evaluating

the ability of bioremediation to reduce the adverse effects of contaminants in soil.

Miadokova E, Vlckova V, Podstavkova S, Slaninova M, Vlcek D. **Unicellular green alga Chlamydomonas reinhardtii as an activation system for 2-aminofluorene.** Environ Mol Mutagen 1998;31(4):383-9.
BIOSIS COPYRIGHT: BIOL ABS. Despite the promutagenic/procarcinogenic potential, polycyclic aromatic amines ore widely spread in the environment. Biotransformation of the polycyclic aromatic amine 2-aminofluorene (2-AF) was proved in mammals and higher plants. The algal cell/microbe coincubation assay is an additional system that complemented those proved in mammals and higher plants, useful for detection and conversion of environmental promutagens, mainly in aquatic environments. The unicellular green algae may be a good activating system in coincubation assays in that the algal cells exist as a natural system. To increase the effectiveness of this metabolizing system, different modifications of the standard experimental procedure were conducted. Algae can accumulate and metabolize promutagenic pollutants, some of which may differ from those activated by the animal microsome metabolizing system (59 mix) and by the plant cell/microbe coincubation assay. 2-AF was activated in the algal cell/ microbe coincubation assay in which wild-type Chlamydomonas reinhardtii cells were used as an activating system and the bacteria Salmonella typhimurium TA98, YG1024, and yeast Saccharomyces cerevisiae D7 as the genetic indicator organisms. It was converted to the mutagenic product(s) for the strain YG1024, but the strain TA98 did not exhibit any increase in the mutant yield of His+ revertants. Consequently, metabolites from 2-AF are substrates for O-acetyltransferase. A direct comparison of algal 2-AF activation with mammalian activation system (S9 mix) proved the higher activity of mammalian microsome system (S9 mix). After the combination of both activation systems, a slight synergetic effect was found. Although the genetic endpoints induced by 2-AF using both modifications of the algal cell/S. cerevisiae coincubation assay and those obtained in intact yeast cells were similar at the equitoxic concentrations, 2-AF activation by the algal supernatant slightly increased the genetic endpoints studied.

Mitchelmore CL, Chipman JK. **DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring.** Mutat Res 1998;399(2):135-47.
This review considers the potential for DNA strand breaks, particularly as measured by the comet assay, to act as a biomarker of genetic toxicity in fish and other aquatic species. The background need for such biomarkers is introduced in relation to carcinogenicity, reproductive effects and other adverse effects of pollution. Sensitive measurements of DNA strand breakage can be achieved, e.g., by alkaline elution, alkaline unwinding or by single cell gel electrophoresis (comet) techniques. The DNA damage can be a reflection, not only of direct strand breakage, but also of alkali-labile sites and of repair enzyme-mediated breakage (i.e., is non-specific). A range of genotoxic chemicals (both with and without the requirement for metabolic activation) give positive effects in various cell types of vertebrate and invertebrate aquatic species, following in vitro and in vivo exposures under laboratory conditions. A limited number of analyses of organisms exposed to polluted waters or sediments in the field have implicated DNA strand breakage as a relatively sensitive, rapid and broad specificity indicator of genotoxic pollutant exposure. The comet assay deserves further exploitation to assess inter-individual and inter-cell variability in response to pollutants and naturally occurring genotoxic stimuli, and to assess the persistence of these effects.

Negri A, Llewellyn L. **Comparative analyses by HPLC and the sodium channel and saxiphilin 3H-saxitoxin receptor assays for paralytic shellfish toxins in crustaceans and molluscs from tropical North West Australia.** Toxicon 1998;36(2):283-98.
The increased frequency and distribution of red tides requires the development of high-throughput detection methods for paralytic shellfish toxins (PST). Community ethics also requires that there be a reduced reliance upon the standard mouse bioassay. A biomolecular assay such as the sodium channel 3H-saxitoxin binding assay can satisfy both of these requirements but may be compromised by cross-reactivity with the structurally unrelated tetrodotoxins (TTX). This study utilised the sodium channel assay but also an alternative 3H-saxitoxin binding assay based upon a saxiphilin isoform from the centipede Ethmostigmus rubripes to screen for PSTs. Saxiphilin is a novel transferrin which binds saxitoxin (STX) but differs from the sodium channel in not having any measurable affinity for TTX. A detailed analysis of toxin composition was achieved by high performance liquid chromatography (HPLC). Various crustaceans and molluscs accumulate PSTs and TTX, thus proving useful biomarkers for these toxins in

their immediate environment and an ideal challenge to the detection and analysis of PSTs in this presumptive screening program. Also, there has been little investigation of PSTs in invertebrates from the Indian Ocean so this region was selected to extend our knowledge of the distribution of these toxins. 190 crabs and shellfish encompassing 31 species were collected from reefs along the North-West Australian coast and tested for PSTs and TTX by sodium channel and saxiphilin bioassays as well as HPLC. PSTs were detected in 18 species of crabs and shellfish of the 31 species tested. Eight of these species have not been previously described as toxic, these being the crabs Euzanthus exsculptus, Lophozozymus octodentatus, Metopograpsus frontalis, Pilumnus pulcher, Platypodia pseudogranulosa and Portunus pelagicus, and the molluscs Tectus fenestratus and Trochus hanleyanus. By HPLC, only one or both of STX and decarbamoyl-STX was detected in any extract. Some extracts markedly inhibited 3H-saxitoxin binding by the sodium channel but not by saxiphilin. The close agreement between toxin quantification by the PST specific methods of HPLC and the saxiphilin bioassay is indicative that the additional toxicity detected by the sodium channel assay is TTX.

Obroucheva NV, Bystrova EI, Ivnov VB, Antipova OV, Seregin IV. **Root growth responses to lead in young maize seedlings.** Plant Soil 1998;200(1):55-61.
BIOSIS COPYRIGHT: BIOL ABS. This work was undertaken to follow the appearance and development of symptoms of lead toxicity in growing roots of seedlings. The effects of lead nitrate (10-2-105 M) were studied on the roots of maize (Zea mays) seedlings, cvs. Diamant and Sterling. The roots were grown on filter paper either on glass in trays or in large Petri dishes. The following characteristics of root growth were studied: seed germination, length of primary and seminal roots, number of seminal and lateral roots, length of branching zone, length of meristem and fully-elongated cells and the number of fully-elongated cells along the daily length increment. 10-2 M lead nitrate exerted a clear toxic effect on root elongation just after radicle emergence; its influence on shoot growth was weak. However 10-2 M Pb solution did not affect either radicle emergence itself or seminal root emergence, which can be explained by the impermeability of seed testa to lead salt. The inhibitory effect of 10-3 M lead nitrate appeared a day later and was not as toxic: the growth of primary and seminal roots proceeded at lower rate due to a partial inhibition of cell division and cell elongation in them. 10-3 M lead nitrate modified the root system morphology: it exerted no effect on the emergence of lateral roots and their number, but induced a more compact distribution of lateral roots along a shorter branching zone due to a reduced length of mature cells in the primary root. As a result of the more prominent inhibition of primary root growth, a shorter branching zone with more compactly located lateral roots occupied a position much closer to the root tip than in roots grown without the influence of lead.

Pedersen F, Bjornestad E, Andersen HV, Kjolholt J, Poll C. **Characterization of sediments from Copenhagen Harbour by use of biotests.** Water Sci Technol 1998;37(6-7):233-40.
BIOSIS COPYRIGHT: BIOL ABS. The potential environmental hazard of sediment samples from Copenhagen Harbour was investigated by a combination of chemical analyses and biological tests. The chemical analyses comprised determination of the content of heavy metals, polycyclic aromatic hydrocarbons and polychlorinated biphenyls, and the biological tests comprised whole sediment bioassays with the amphipod Corophium volutator, testing of a sediment suspension with the Microtox Solid Phase test, and testing of sediment pore water by the copepod Acartia tonsa and the alga Skeletonema costatum. For all sediment samples, the concentrations of contaminants exceeded the Probable Effect Levels and toxic effects should therefore be expected. However, various degrees of toxicity were determined by the biotests with the Microtox Solid Phase test system being the most sensitive, the amphipod and the copepod being intermediately sensitive, and no toxicity of the pore water was registered to the algae. Moreover, no direct correlation between the content of the contaminants in the sediment samples and the registered toxicity could be established. It is therefore concluded that the combination of biotesting and chemical analysis is a valuable tool for evaluating the environmental risks of sediments.

Ralph S, Petras M. **Caged amphibian tadpoles and in situ genotoxicity monitoring of aquatic environments with the alkaline single cell gel electrophoresis (comet) assay.** Mutat Res 1998;413(3):235-50.
In previous studies we demonstrated that indigenous amphibian tadpoles are suitable organisms for monitoring small bodies of water (e.g., creeks, ponds, and drainage ditches) using the alkaline single cell gel electrophoresis

(SCG) or 'comet' assay. This approach involves detection, under alkaline conditions, of cell DNA fragments which on electrophoresis migrate from the nuclear core, resulting in a 'comet with tail' formation. However, although often plentiful, tadpoles are not present in all aquatic environments. Both larger bodies of water (e.g., lakes and rivers) and those impacted upon heavily by man (e.g., bodies of water near industrial sites, on landfills, and in urban areas) often do not support amphibian tadpole populations. An alternative approach to the collection of indigenous tadpoles is to place caged tadpoles at these sites for short term exposures to environmental contaminants. To determine the feasibility of such an approach, Rana clamitans (green frog) and Bufo americanus (American toad) tadpoles were housed in cages at 11 sites in southwestern Ontario (Canada). In a preliminary experiment, we found that tadpoles caged at a polluted reference site (Tallgrass Prairie Heritage Park in Windsor, Ontario) for either 7 or 14 days showed significant ($P < 0.05$) increases in DNA damage, relative to tadpoles caged in the laboratory in dechlorinated water. As a result we routinely used a 7 day exposure time. Significantly ($P < 0.05$) increased levels of DNA damage, relative to their controls, were observed in tadpoles caged at three sites along two creeks draining a large petrochemical installation south of Sarnia, Ontario; at two sites in the Tallgrass Prairie Heritage Park; and at a site along the Ecarte Channel which is part of the St. Clair River. The DNA damage levels of animals caged in Lake St. Clair, in the Trenton Channel of the Detroit River, at a landfill site, and in two creeks in the city of Windsor did not differ significantly ($P > 0.05$) from their controls. This study demonstrates that caged tadpoles are suitable for monitoring most bodies of fresh water, particularly those aquatic habitats mentioned above where indigenous tadpoles are not present. A combined approach of collecting indigenous tadpoles and using caged tadpoles should provide a sensitive system for aquatic genotoxicity monitoring.

Ralph S, Petras M. **Comparison of sensitivity to methyl methanesulphonate among tadpole developmental stages using the alkaline single-cell gel electrophoresis (comet) assay.** Environ Mol Mutagen 1998;31(4):374-82.
In a previous study, we demonstrated that tadpoles are suitable organisms for monitoring small bodies of water (e. g., creeks, ponds, and drainage ditches) for genotoxicity using the alkaline single-cell gel DNA electrophoresis (SCG) or comet assay [Ralph and Petras, 1997]. This approach involves detection, under alkaline conditions, of cell DNA fragments which on electrophoresis migrate from the nuclear core, resulting in a comet with tail formation. In this initial study, most of the tadpoles collected were in the early stages of larval development, but this is not always possible. The present study evaluated the sensitivity of tadpoles, at different stages of larval development, to a range of concentrations of the genotoxicant methyl methane-sulphonate (MMS). Four specific phases of Rana clamitans (green frog) larval development were examined: first-year limbless tadpoles (Stage I as defined by Taylor and Kollros [1946]), second-year limbless tadpoles (Stages II-III), second-year tadpoles with only hindlimbs (Stages X-XVIII), and second-year tadpoles with all four limbs evident and a tail undergoing resorption (Stages XXII-XXIII). Twenty-four hour exposures to MMS of tadpoles in the three earliest phases produced a significant ($P < 0.01$) added variance component among tadpoles for DNA damage and there were significant increases ($P < 0.05$) in the length:width ratios of the DNA patterns at concentrations as low as 1.56 mg/l. However, tadpoles in the last phase studied (both pairs of limbs present) showed no significant ($P > 0.05$) added variance component and no significant increases ($P> 0.05$) in DNA damage upon exposure to any of the MMS doses tested. A nested ANOVA indicated that, for each of the tested concentrations of MMS, but not the dechlorinated water control, there was significant heterogeneity ($P < 0.05$) in DNA damage when tadpoles of all four phases studied were compared. However, when tadpoles of the lost phase of development were removed from the comparison, there was no significant heterogeneity ($P > 0.05$) among tadpoles of the remaining three phases. Possible reasons for this insensitivity to MMS as animals enter the metamorphic climax were considered. The results indicate that pooling of the early tadpole phases of R. clamitans for SCG environmental genotoxicity biomonitoring is acceptable.

Rojickova-Padrtova R, Marsalek B, Holoubek I. **Evaluation of alternative and standard toxicity assays for screening of environmental samples: selection of an optimal test battery.** Chemosphere 1998;37(3):495-507.
BIOSIS COPYRIGHT: BIOL ABS. Six muniaturized alternative assays (called microbiotests) and three standard toxicity tests were used for a comparative study based on the evaluation of acute toxicity of fifty environmental samples. The test species used in the alternative assays were microalga Raphidocelis subcapitata, crustaceans Thamnocephalus platyurus and Ceriodaphnia dubia, rotifer Brachionus calyciflorus, protozoan Spirostomum ambiguum and bacterium Vibrio fischeri. The standard toxicity tests utilized microalga Raphidocelis subcapitata,

crustacean Daphnia magna, and fish Poecilia reticulata as the test organisms. The study compared the ability of bioassays to detect acute toxicity, relative sensitivity of the six microbiotests with regard to three standard toxicity tests, and similarity in their sensitivity to fifty samples. Algal bioassays were the most sensitive tests. Ceriodaphnia dubia and Spirostomum ambiguum detected acute toxicity in the majority of samples (in 6272%). Vibrio fischeri showed a specific sensitivity pattern that was associated neither with algal nor with animal tests. The other species formed the least sensitive organisms, having similar sensitivities. A battery of three to four alternative assays was selected on the basis of the statistical analyses, sensitivity comparisons and general conditions for the selection of a test battery member like, incorporation of different trophic levels or complementation of assays in a battery. Therefore, miniaturized algal assay, rotifer or crustacean microbiotest, bacterial test and possibly protozoan microbiotest could represent an optimal battery of alternative assays for the toxicity evaluation of fifty environmental samples presented in this study.

Sabaliunas D, Lazutka J, Sabaliuniene I, Sodergren A. **Use of semipermeable membrane devices for studying effects of organic pollutants: comparison of pesticide uptake by semipermeable membrane devices and mussels.** Environ Toxicol Chem 1998;17(9):1815-24.
BIOSIS COPYRIGHT: BIOL ABS. Uptake of four pesticides-the organochlorines chlordane and endosulfan and the synthetic pyrethroids fenvalerate and allethrin-by triolein-containing semipermeable membrane devices (SPMDs) and by the lake mussel Anodonta piscinalis was studied in a laboratory continuous-flow system. Uptake of the analytes by the SPMDs and mussels was linear during the exposure period of 20 d. These kinetic data were used to calculate the first-order uptake rate constants. On a SPMD-whole body basis, the uptake rates were 3.5 to 5.5 times higher in the membrane devices than in the organisms. The synthetic pyrethroids were sampled at lower rates than the organochlorines, and this difference may be attributed to the larger molecular dimensions of the pyrethroids rather than analyte molecular weight and lipophilicity, which were similar for all test compounds. Because of the disparate sampling rates, concentration factors of analytes differed between SPMDs and mussels. However, the percent composition (ratios) of analytes in SPMDs and in mussels was similar, which indicates that SPMDs may serve as good surrogates for aquatic organisms with respect to the discriminatory uptake of hydrophobic chemicals. Semipermeable membrane device dialysate, mussel extract, as well as two artificial mixtures of the four pesticides were tested with standard toxicity and genotoxicity tests, including Microtox (inhibition of bacterial luminescence), Daphtoxkit, and Rotoxkit (toxicity tests with freshwater invertebrates Daphniapulex and Brachionus calyciflorus, respectively), and sister chromatid exchange in human lymphocytes in in vitro assay. Results of these tests suggest that integration of the SPMD technique and bioassays may be a valuable approach for the assessment of levels and effects of bioavailable hydrophobic pollutants.

Sae-Ma B, Meier PG, Landrum PF. **Effect of extended storage time on the toxicity of sediment-associated cadmium on midge larvae (Chironomus tentans).** Ecotoxicology 1998;7(3):133-9.
BIOSIS COPYRIGHT: BIOL ABS. The effect of the duration of spiked sediment storage on cadmium toxicity was studied. Sediment samples were spiked with cadmium to obtain concentrations of 0.6, 16.0, 29.0 and 53.0 mug Cd per sediment (dry weight). The spiked sediment was then stored in sealed plastic containers at 4~ C in the dark. Sediment bioassays, using Chironomus tentans, were conducted immediately and at periodic intervals for up to 4 months. Though the levels of cadmium in the bulk sediment samples from different stored periods were not significantly different. different toxicity levels to C. tentans were observed. The toxicity was significantly different between subsequent storage times. There was a significant decrease in the bioaccumulation factor (BAF) values with extended storage times, indicating a reduction in the bioavailability of cadmium. This study suggests that the storage of spiked sediment used in sediment toxicity study can influence the results.

Scott A. **Environment-accident index: validation of a model.** J Hazardous Materials 1998;61(1-3):305-12.
BIOSIS COPYRIGHT: BIOL ABS. Authorities and industry often have difficulties knowing what to focus on when it comes to risk assessment in the handling of chemicals. Hence, there is a requirement for a tool to facilitate this work. The Environment-Accident-Index (EAI) is proposed as such a tool. EAI is a simple model that gives guidance as to the identification and quick ranking of the kind of assessment to be performed. EAI is built on three parts: the first part contains information on the acute toxicity to aquatic organisms, the second part concerns the transported

or stored amount of the chemical and the third part deals with chemical mobility. For example, the mobility part contains chemical-physical properties of the substance and those of the surrounding environment, such as the possibility of soil penetration and depth and mobility of the groundwater. The purpose of the EAI is to be an implement, to be used by authorities and industry, when planning for the storage and transportation of chemicals, amongst other uses. The model is intended to be simple to facilitate and increase the use of EAI, The results show that EAI is quite capable of becoming a useful tool for ranking different hazards and that EAI is a good basis for further development of the model. The results also show that there is a lack of environmental data available from chemical accidents and that a better system for environmental follow-up of chemical accidents would have facilitated and given a better foundation to the evaluation. The evaluation has been performed by Asa Scott, Defence Research Establishment, by order of the Swedish Rescue Services Agency and the Swedish Environmental Protection Agency. An evaluation group has supported and guided the work. The evaluation group has, besides the Swedish Rescue Services Agency, the Swedish Environmental Protection Agency and Defence Research Establishment included experts from the National Chemicals Inspectorate in Sweden and the Association of Swedish Chemical Industries. The paper is an evaluation of EAI and discusses the present usefulness of the index and conceivable changes for the future.

Steevens JA, Vansal SS, Kallies KW, Knight SS, Cooper CM, Benson WH. **Toxicological evaluation of constructed wetland habitat sediments utilizing Hyalella azteca 10-day sediment toxicity test and bacterial bioluminescence.** Chemosphere 1998;36(15):3167-80.
BIOSIS COPYRIGHT: BIOL ABS. A toxicological evaluation was conducted on wetland habitats created as a result of run-off from agricultural areas. These temporary wetlands were created by using drop pipes as a means of reducing erosional cutting in agricultural fields. Toxicity bioassays utilizing bacterial bioluminescence and Hyalella azteca were used to assess sediment pore water and whole sediment, respectively. Inhibition of bacterial bioluminescence was initially used to determine relative toxicities of pore water from ten wetland sites. Constructed wetland sites were compared to the University of Mississippi Biological Field Station, a relatively pristine reference site. The H. azteca ten day sediment toxicity test was utilized to assess sediment from four selected sites using survival and growth as toxicological endpoints. Results from the toxicological evaluation, along with extensive ecological evaluations, were used to assess the best approach for implementation of temporary wetland habitats with existing agricultural practices.

Steinert SA, Streib-Montee R, Leather JM, Chadwick DB. **DNA damage in mussels at sites in San Diego Bay.** Mutat Res 1998;399(1):65-85.
Identification and assessment of introduced and other toxicants is crucial to any comprehensive study of contaminants within the marine environment. The relationship between DNA single-strand breaks and the exposure of marine organisms to environmental contaminants was examined at sites in San Diego Bay, CA. A comprehensive assessment of the extent and consequences of marine environmental contamination in the area of Naval Station San Diego was conducted in the summer of 1995. The study addressed contamination sources, distributions, concentrations, transport, sediment-water exchange, biological effects, and degradation. The biological effects portion of the study (this paper) included contaminant bioaccumulation, organismal growth, and the determination of DNA single-strand breaks using the Comet assay. DNA damage was determined in hemocytes collected from deployed and resident mussels, Mytilus edulis, at six stations in and around the Naval Station San Diego. Deployed mussels were exposed on station for approximately 30 days in plastic mesh bags, placed 1 m above the bottom. Hemocyte samples were collected on days 0, 12, and 32. It was found that stations exhibiting the extremes of contaminant exposure, both highest and lowest concentrations, were easily identified using growth and DNA damage measurements. Sediment chemistry and bioaccumulation data indicated, Hg, Cu, and Zn, to be the most notable contaminants. The Comet assay, and in particular germ cell DNA damage determinations, were found to respond rapidly to station contaminants. Results from this study and an earlier 1993 study suggest that the non-sediment associated effects observed at one station may have been the result of the photoactivation of accumulated PAHs.

Sunahara GI, Dodard S, Sarrazin M, Paquet L, Ampleman G, Thiboutot S, Hawari J, Renoux AY. **Development of**

a soil extraction procedure for ecotoxicity characterization of energetic compounds. Ecotoxicol Environ Saf 1998;39(3):185-94.
The acetonitrile-sonication extraction method (US EPA Method 8330) associated with aquatic-based toxicity tests was examined to study the ecotoxicity of energetic substances in soil. Three studies were carried out: (1) toxicological characterization of different energetic substances to select a representative toxicant and to validate the choice of bioassays; (2) choice of an appropriate solvent to transfer acetonitrile extracts to the bioassay incubation media; and (3) optimization of Method 8330 using soil samples spiked with the toxicant. Initial studies indicated that pure 2,4,6-trinitrotoluene (TNT) was toxic to Vibrio fischeri [Microtox; IC50 (15 min) of 4.2 microM], whereas RDX was less toxic (IC20 = 181 microM) and HMX was not toxic up to its limit of water solubility (< 22 microM). Selected pure TNT metabolites were less toxic than TNT. Similar results were found using the 96-h Selenastrum capricornutum growth inhibition test. The toxicity of pure TNT in different solvents (acetonitrile, acetone, and DMSO) and that from Method 8330-extracted TNT-spiked soil samples were compared to TNT dissolved in water. Data indicated that DMSO was the most appropriate solvent to transfer the acetonitrile extracts. A modified Method 8330 may be used in conjunction with bioassays and chemical analyses to examine the ecotoxicity of soils contaminated with energetic substances.

Svenson A, Viktor T, Remberger M. **Toxicity of elemental sulfur in sediments.** Environ Toxicol Water Qual 1998;13(3):217-24.
BIOSIS COPYRIGHT: BIOL ABS. Elemental sulfur occurs naturally in marine and limnic sediments. Elemental sulfur, brought in solution in aqueous media by using organic solvents such as methanol as carrier solvent, was toxic in a bacterial luminescence test, known as the Microtox test. Previously, it has been shown that the toxicity in the luminescence test of whole sediments also was correlated to i.a. elemental sulfur using multivariate statistical analysis. Organic solvent extracts of sediments obtained in receiving waters of effluents from a pulp and paper mill was toxic in the luminescence test, and using a toxicity evaluation procedure, the toxic substance was identified as octameric cyclic sulfur, S8. The substance dominated the toxicity in extracts of both a contaminated sediment and a sediment from a control area. Since the toxicity in the Microtox test of aqueous solutions of S8 decreased upon storage, a conversion process of the toxic form was indicated. Acute toxicity of S8 was not limited to the luminescent bacteria in the Microtox test, but was observed in tests with fish larvae if tested with the transient form of elemental sulfur. Tests of acute toxicity with zebra fish and perch larvae were responsive to elemental sulfur. Probably, the toxic form of elemental sulfur is the single cyclic octamer, that due to low aqueous solubility, binding to particulate sediment material or aggregation is converted into a nontoxic form. Acute toxic effects may occur in sulfur containing sediments of varying redox potentials or where elemental sulfur deposits are turbated.

Tatara CP, Newman MC, McCloskey JT, Williams PL. **Use of ion characteristics to predict relative toxicity of mono-, di- and trivalent metal ions: Caenorhabditis elegans LC50.** Aquatic Toxicol 1998;42(4):255-69.
BIOSIS COPYRIGHT: BIOL ABS. Predictive models for relative toxicity of divalent metal ions using ion characteristics have been produced with both Microtox, a 15 min microbial bioassay, and the 24 h Caenorhabditis elegans bioassay. Relative toxicity of mono-, di- and trivalent metal ions has also been successfully modeled using ion characteristics with the Microtox bioassay. This study extends this approach to include longer exposure durations (24 h) and a more complex organism (metazoan). Twenty-four-hour LC50s (expressed as total and free ion concentrations) for the free-living soil nematode, C. elegans, were determined for Li, Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Cd, Cs, Ba, La, and Pb in an aqueous medium. Relative metal toxicity was predicted with least squares linear regression and several ion characteristics. Toxicity was most effectively predicted (r2 = 0.85) with a two-variable model containing log KOH (where KOH is the first hydrolysis constant) and chim2r (the covalent index). The first hydrolysis constant reflects a metal ion's tendency to bind to intermediate ligands such as biochemical groups with O donor atoms, while chim2r reflects binding to soft ligands such as those with S donor atoms. The use of LC50s based on free ion concentrations did not significantly improve model fit. The results of this study are consistent with earlier models generated with Microtox data, with the exception of barium, which was much more toxic to C. elegans than would be predicted from the model. We conclude that, with thoughtful application, ion characteristics can be used to predict the relative toxicity of metal ions that vary widely in both valence and binding tendency.

Teisseire H, Couderchet M, Vernet G. **Toxic responses and catalase activity of Lemna minor L. exposed to folpet, copper, and their combination.** Ecotoxicol Environ Saf 1998;40(3):194-200.
Toxicity of copper and folpet--two fungicides widely used on grape--was evaluated on Lemna minor L., a sensitive aquatic weed regularly used for (eco)toxicological studies. Toxicity assessments were based on inhibition of growth and chlorophyll content of L. minor cultures after 7 days. IC10, IC50, and IC90 were determined for both compounds alone and were respectively, 0.03, 0.16, and 0.95 mg liter-1 for copper and 1.20, 7.50, and > 40 mg liter-1 for folpet. When both compounds were combined, the response of L. minor depended on the initial folpet concentration. Indeed, a slight synergy was observed for 5 mg liter-1 folpet, while at folpet concentrations of 20 to 35 mg liter-1, the two fungicides were antagonists. The antagonism was positively correlated with folpet concentration. Antagonism between Cu and folpet could not be explained by a reduced bioavailability of Cu since concentration of free copper in the mixture did not depend on the presence of folpet. One physiological defense response elicited by copper in plants is an increase in catalase activity. Copper and folpet stimulated catalase activity and changes in the activity of the enzyme could not account for the synergy but possibly for the antagonism. Nevertheless, catalase activity increase significantly after a 24-h exposure to 25 micrograms liter-1 of copper. The use of this property as a rapid and sensitive biomarker to monitor the toxicity of xenobiotics alone or in combination and of environmental water is discussed.

Vaes WH, Ramos EU, Verhaar HJ, Hermens JL. **Acute toxicity of nonpolar versus polar narcosis: is there a difference?** Environ Toxicol Chem 1998;17(7):1380-4.
BIOSIS COPYRIGHT: BIOL ABS. Narcosis I and II chemicals exhibit different lethal body burdens (LBBs) and acute toxicities (median lethal concentrations (LC50's)) in fish, when compared with their log n-octanol-water partition coefficient (Kow). The higher toxicity of polar narcosis chemicals has often been suggested to be related to the polar group in these molecules. Recently we showed that membrane (L-alphadimyristoyl phosphatidyl-choline (DMPC))-water partition coefficients (log KDMPC) are higher than log Kow for narcosis II chemicals. Using log KDMPC to model the acute toxicities to fish (log LC50) of the two classes of chemicals together, gives one high-quality quantitative structure-activity relationship (R2 = 0.98, Q2 = 0.97). In addition, assuming that the apolar fat-water partition coefficient behaves like the n-octanol-water partition coefficient, differences in LBB can also be explained. Thus, using log KDMPCs, all earlier reported quantitative differences between narcosis I and II chemicals can be explained.

Walker CH. **The use of biomarkers to measure the interactive effects of chemicals.** Ecotoxicol Environ Saf 1998;40(1-2):65-70.
Biomarker assays that provide measures of the toxic effects of chemicals on key organisms are of particular interest in ecotoxicology and environmental risk assessment. Typically, such assays provide measures of the molecular mechanisms that underlie toxicity (e.g., inhibition of brain acetylcholinesterase activity by organophosphorus insecticides and retardation of the vitamin K cycle by anticoagulant rodenticides). They are particularly valuable for detecting and quantifying toxicity where organisms are exposed to mixtures of compounds and for identifying cases of potentiation. In birds, inhibition of brain acetylcholinesterase activity can provide an index of potentiation of organophosphorus and carbamate insecticides by other pesticides. Inhibition of serum butyrylcholinesterase also is very useful as a nondestructive assay but is not simply related to inhibition of brain acetylcholinesterase. Assays for DNA damage can indicate where there is an increase in the rate of activation of carcinogens and mutagens due to induction of the cytochrome P450 system. Assays for blood levels of retinol (vitamin A) and thyroxine can establish thyroxine antagonism by metabolites of 3,3,4,4-tetrachlorobiphenyl. Assays for changes in levels of clotting protein in serum can give an indication of the effect of mixtures of anticoagulant rodenticides on the vitamin K cycle. The interactive effects of mixtures of pesticides in the field are starting to be investigated by this approach (e.g., a recent study of the combined action of malathion and prochloraz in the red-legged partridge).

White DC, Flemming CA, Leung KT, Macnaughton SJ. **In situ microbial ecology for quantitative appraisal, monitoring, and risk assessment of pollution remediation in soils, the subsurface, the rhizophere and in biofilms.** J Microbiol Methods 1998;32(2):93-105.
BIOSIS COPYRIGHT: BIOL ABS. Numerous studies have established a relationship between soil, sediment,

surface biofilm and subsurface contaminant pollution and a marked impact on the in situ microbial community in both microcosms and in the field. The impact of pollution on the in situ microbial community can now be quantitatively measured by molecular 'fingerprinting' using 'signature' biomarkers. Such molecular fingerprinting methods can replace classical microbiological techniques that relied on isolation and subsequent growth of specific microbes from the in situ microbial community. Classical methods often revealed less than 1% of the extant microbial communities. Molecular fingerprinting provides a quantitative measure of the in situ viable microbial biomass, community composition, nutritional status, relative frequency of specific functional genes, nucleic acid polymers of specific microbes, and, in some cases, the community metabolic activity can be inferred. Current research is directed at establishing correlations between contaminant disappearance, diminution in toxicity, and the return of the viable biomass, community composition, nutritional status, gene patterns of the in situ microbial community towards that of the uncontaminated soil, sediment or subsurface material with the original uncontaminated microniche environments. Compared to the current reliance on disappearance of pollutants and associated potentially toxic products for detection of effective and quantitative bioremediation, assessment of the in situ microbial community will be an additional and possibly more convincing risk assessment too). The living community tends to accumulate and replicate toxic insults through multiple interactions within the community, which may then effect viable biomass, community composition, nutritional status, community metabolic activities, and specific nucleic acid polymer patterns.

Wilson JT, Pascoe PL, Parry JM, Dixon DR. **Evaluation of the comet assay as a method for the detection of DNA damage in the cells of a marine invertebrate, Mytilus edulis L. (Mollusca: Pelecypoda).** Mutat Res 1998;399(1):87-95.
The potential application of the comet assay for monitoring the effect of DNA damaging agents on the marine mussel, Mytilus edulis (an important pollution indicator organism), was explored. A detailed investigation of the baseline levels of single-strand breaks in isolated gill cells, and how they were affected by age/size of animal, time since collection, feeding regime, in vivo vs. in vitro exposure conditions, and by antioxidant supplementation was undertaken. The level of cometing in untreated controls was found to be highly variable over time (fluctuations between low and very high DNA damage occurred over just 14 days post collection). No difference was observed between age/size and feeding regime of the animals. On exposure to 0, 100, 500 and 1000 microM H2O2, it was observed that the in vitro exposure produced a markedly more homogeneous dose response compared to the in vivo studies (where gill cells were exposed as a tissue). An important finding of our research was the effect of prior supplementation of the animals' diet with 1 mg/ml alpha-tocopherol acetate (vitamin E compound), which resulted in a marked reduction in the levels of DNA damage expressed by the negative controls, without influencing the actual response to H2O2 (0, 5, 25, and 100 microM) and N-nitrosodimethylamine, NDMA (0, 5, 25, and 100 mM). The effect of vitamin E supplementation was to increase the sensitivity of the comet assay at the lower end of the dose range. This study demonstrated the potential application of the comet assay to the gill cells of the mussel, M. edulis. Although preliminary findings suggest that antioxidant supplementation can improve the sensitivity of the assay by lowering the baseline damage in untreated animals, our conclusion is that the assay has more potential for use in an in vitro context for the screening of agents destined for release or disposal into the marine environment.

Zhao YH, Ji GD, Cronin MT, Dearden JC. **QSAR study of the toxicity of benzoic acids to Vibrio fischeri, Daphnia magna and carp.** Sci Total Environ 1998;216(3):205-15.
The toxicities of benzoic acids to Vibrio fischeri, Daphnia magna and carp were measured. The results showed that the toxicity to V. fischeri and Daphnia decreased in the order of bromo > chloro > fluoro approximately equal to aminobenzoic acids. The toxicity of substituted benzoic acids to carp and Daphnia was much lower that to V. fischeri. The results also showed that the toxicity of benzoic acids to Daphnia decreased as the pH increased. It is suggested that ionized and non-ionized forms have different toxic responses. The non-ionized form may play an important role in toxicity because the toxicity of benzoic acids to Daphnia greatly decreases as the pH increases. The toxicity of benzoic acids to Daphnia may operate through non-polar narcosis, based on the regression results between the toxicities and partition coefficients (log P) and apparent partition coefficients (log D). However, toxicity cannot be predicted from non-polar baseline models because the ionized and non-ionized form of benzoic acids have different contributions to toxicity. Compared with the single descriptors, the prediction of toxicity of the benzoic

acids was improved remarkably by using log P with pKa and log P with ELUMO. For the toxicity of benzoic acids to V. fischeri, it is suggested that the toxic mechanism may be different from the mechanism in Daphnia and carp. A probable reason is that V. fischeri is a unicellular organism with low lipid content, and hence both ionized and non-ionized forms of benzoic acids can easily cross the cell membrane and contribute to toxicity.

GENOTOXICITY AND MUTAGENESIS

Adler ID, Bootman J, Favor J, Hook G, Schriever-Schwemmer G, Welzl G, Whorton E, Yoshimura I, Hayashi M. **Recommendations for statistical designs of in vivo mutagenicity tests with regard to subsequent statistical analysis.** Mutat Res 1998;417(1):19-30.
A workshop was held on September 13 and 14, 1993, at the GSF, Neuherberg, Germany, to start a discussion of experimental design and statistical analysis issues for three in vivo mutagenicity test systems, the micronucleus test in mouse bone marrow/peripheral blood, the chromosomal aberration tests in mouse bone marrow/differentiating spermatogonia, and the mouse dominant lethal test. The discussion has now come to conclusions which we would like to make generally known. Rather than dwell upon specific statistical tests which could be used for data analysis, serious consideration was given to test design. However, the test design, its power of detecting a given increase of adverse effects and the test statistics are interrelated. Detailed analyses of historical negative control data led to important recommendations for each test system. Concerning the statistical sensitivity parameters, a type I error of 0.05 (one tailed), a type II error of 0.20 and a dose related increase of twice the background (negative control) frequencies were generally adopted. It was recommended that sufficient observations (cells, implants) be planned for each analysis unit (animal) so that at least one adverse outcome (micronucleus, aberrant cell, dead implant) would likely be observed. The treated animal was the smallest unit of analysis allowed. On the basis of these general consideration the sample size was determined for each of the three assays. A minimum of 2000 immature erythrocytes/animal should be scored for micronuclei from each of at least 4 animals in each comparison group in the micronucleus assays. A minimum of 200 cells should be scored for chromosomal aberrations from each of at least 5 animals in each comparison group in the aberration assays. In the dominant lethal test, a minimum of 400 implants (40-50 pregnant females) are required per dose group for each mating period. The analysis unit for the dominant lethal test would be the treated male unless the background frequency of dead implants (DI) is so low that multiple males would need to be integrated to meet the minimum observation of one adverse outcome (DI) per analysis unit. A three-step strategy of data analysis was proposed for the cytogenetic assays. Use of negative historical controls was allowed in certain circumstances for interpretation of results from micronucleus tests and chromosomal aberration tests. Copyright 1998 Elsevier Science B.V.

Akiyama N, Alexander D, Aoki Y, Noda M. **Characterization of mutations induced by 300 and 320 nm UV radiation in a rat fibroblast cell line.** Mutat Res 1996;372(1):119-31.
The cytotoxicity, mutagenicity, and mutation specificity associated with ultraviolet (UV) wavelengths of 254, 290, 300 and 320 nanometers (nm) were examined in a rat fibroblast cell line. Cells from the LTK15/CREF cell line were stably infected with the neo gene for forward selection and the herpes-simplex virus thymidine-kinase (HSVtk) gene for reverse selection. Mutations were detected by acyclovir resistance. At 300nm cells were irradiated in the dose range 100 to 250 joules per square meter (J/m2). At 320nm cells were irradiated at a dose range of 3,000 to 5,000J/m2. The cytotoxicity action spectrum closely fitted that for bacteria measured in previous experiments, but was ten times higher at 320nm. The action spectrum for mutagenicity based on the doses needed to induce acyclovir resistant colonies paralleled that for cytotoxicity. At 300nm, but not 320nm, there was a significant increase in large deletions. Some of the G418 sensitive clones contained defects in both neo and HSVtk markers. Polymerase chain reaction analysis showed that the neo gene was intact, but the HSVtk gene was disrupted by large deletions. The frequency of large deletions appeared to increase when cells were irradiated above 300nm. The relative frequencies of tandem double mutations were high at both wavelengths. Complex mutations (multiple events) were higher at 320nm than at 300nm. Most target sites were associated with dipyrimidine or oligopyrimidine sequences, and cytosine repeats were the preferred target. The authors conclude that this assay system provides a valid and useful method for providing important information on the mechanisms of mutagenicity.

Albertini RJ. **The use and interpretation of biomarkers of environmental genotoxicity in humans.** Biotherapy 1998;11(2-3):155-67.

Allaman-Pillet N, Djemai A, Bonny C, Schorderet DF. **Methylation status of CpG sites and methyl-CpG binding proteins are involved in the promoter regulation of the mouse Xist gene.** Gene Expr 1998;7(2):61-73.
The mouse Xist gene is expressed exclusively from the inactive X chromosome and is involved in the initiation of X inactivation. We previously reported that the -1157/+917 region of the Xist promoter was ubiquitously functional in mammalian cells and that experiments in a transient expression system revealed no trans-acting element responsible for the inactive X specific expression of Xist. In somatic tissues, the 5' end of the silent Xist allele on the active X is known to be fully methylated whereas the expressed allele on the inactive X is unmethylated. In the present study we have used a bisulphite genomic sequencing method to evaluate DNA methylation at all cytosines including CpG dinucleotides within the Xist promoter. We report and confirm that methylation of specific sites plays a key role in Xist gene expression. In vitro DNA methylation of the 5'-region drastically reduced transcriptional activity in transiently transfected fibroblasts. Mobility shift assays showed that methylation does not inhibit Xist promoter activity by preventing the binding of transcription factors and that two distinct nuclear proteins bind in a sequence methyl-CpG-specific manner. Therefore, we suggest that Xist repression involves its promoter methylation and two distinct methylated DNA binding proteins.

Andersen D, Dobrzynka MM, Jackson LI, Yu TW, Brinkworth MH. **Somatic and germ cell effects in rats and mice after treatment with 1,3-butadiene and its metabolites, 1,2-epoxybutene and 1,2,3,4-diepoxybutane.** Mutat Res 1997;391(3):233-42.
An investigation was done on the reported species differences in the effects of 1,3-butadiene (106990) (BD) and its metabolites, 1,2-epoxybutene (930223) (EB) and 1,2,3,4-diepoxybutane (298180) (DB), on somatic cells and germ cells of rats and mice using Sprague-Dawley-rats and CD-1-mice. The animals were exposed to BD concentrations of 12.5, 65, and 130 parts per million (ppm) in a whole body exposure system for periods of 6 hours to 4 weeks. Controls were exposed to ambient air. The rats and mice were also injected intraperitoneally with EB doses of 0 to 120mg/kg or DB doses of 0 to 50mg/kg. Following exposures, testicular, bone marrow, and liver cells were isolated and examined for DNA strand breakage in the Comet assay. Both the Giemsa and acridine-orange staining methods were used to analyze bone marrow cells in the micronucleus assay. Unscheduled DNA synthesis (UDS) was determined using scintillation counting in mice injected with 200mg/kg cyclophosphamide (CP), 40 or 80mg/kg EB, and 15 or 30mg/kg DB. Exposure to BD did not significantly affect the tail moments in the liver, bone marrow, and testicular cells of mice. In contrast, EB induced significant increases in tail moments in the bone marrow and testicular cells of mice and the bone marrow cells of rats. DB induced moderate increases in the tail moments in the bone marrow cells, but not testicular cells, of mice and rats. The frequency of micronuclei was significantly increased in the bone marrow cells of both mice and rats exposed to either EB or DB. UDS was observed in the sperm heads of mice exposed to CP, EB, and DB. The authors conclude that CD-1-mice are more susceptible than Sprague-Dawley-rats to the genotoxic effects of EB and DB.

Anderson D, Dhawan A, Yu TW, Plewa MJ. **An investigation of bone marrow and testicular cells in vivo using the Comet assay.** Mutat Res 1996;370(3-4):159-74.
The ability of a computerized image analysis system (COMET) assay to detect DNA destruction in bone marrow somatic and testicular cells of rats was evaluated. Male Sprague-Dawley-rats were treated with cyclophosphamide (50180) (CP), ethyl-methanesulfonate (62500) (EMS), bleomycin (9041934), or ethylene-glycol-monomethyl-ether (109864) (EGME) by gavage. Rats were killed at 2 weeks after treatment for CP, EMS and BLM. For EGME, rats were killed at 2, 5, or 6 weeks. Bone marrow and testicular cells were obtained for COMET assay. The COMET parameters measured were percentage head DNA content, tail length, and tail moment. CP at 50 and 100mg/kg produced DNA destruction in bone marrow and testicular cells. EMS at 100, 200, and 300mg/kg produced slight increases in DNA destruction in bone marrow, and more marked damage in testicular cells. Bleomycin at 50, 100, and 150mg/kg produced small increases in damage to DNA in bone marrow and testicular cells. EGME at 500, 1,000, and 1,500mg/kg caused increased damage to DNA in bone marrow and testicular cells, and a sharp decrease in percentage head DNA content. The animals were most sensitive to CP and EGME. The authors

conclude that COMET assay appears useful for measuring damage to DNA and its persistence, and for comparing the sensitivity of different target organs in-vivo.

Arimochi H, Kinouchi T, Kataoka K, Kuwahara T, Ohnishi Y. **Activation of 1-nitropyrene by nitroreductase increases the DNA adduct level and mutagenicity.** J Med Invest 1998;44(3-4):193-8.
BIOSIS COPYRIGHT: BIOL ABS. 1-Nitropyrene (1-NP) is a mutagenic nitro compound in the environment. We studied correlations between the mutagenicity of 1-NP for three strains of Salmonella typhimurium, the activity of bacterial nitroreductases and the amount of 1-NP-derived DNA adducts. Bacterial strains used in this study were S. typhimurium strains TA98, nitroreductase-less mutant TA98NR and YG1021 carrying a nitroreductase-producing plasmid. The mutagenicity of 1-NP was measured using the Ames assay, and the nitroreductase activities of these strains were assayed by quantification of 1-aminopyrene produced from 1-NP. The DNA adducts were measured by the 32P-postlabeling method. Among the three bacterial strains, strain YG101 was the highest in mutagenicity of 1-NP, the nitroreductase activity and the DNA adduct level. However, S. typhimurium strain TA98NR had the lowest values of these three parameters. Nitroreductase activity, DNA adduct level and mutagenicity were strongly correlated with each other. These results indicate that bacterial nitroreductase plays an important role in forming the DNA adducts, and that the higher the adduct level the higher the level of mutagenicity.

Asakura S, Sawada S, Sugihara T, Daimon H, Sagami F. **Quinoline-induced chromosome aberrations and sister chromatid exchanges in rat liver.** Environ Mol Mutagen 1997;30(4):459-67.
The effects of quinoline (91225) exposure on chromosome aberrations and sister chromatid exchanges (SCEs) were studied. Male Fischer-344-rats were intubated with a quinoline dose of 200mg/kg or an 8-hydroxyquinoline (148243) (8HQ) dose of 500mg/kg and killed 4 to 48 hours later. In the dose response study, rats were intubated daily with quinoline doses of 25 to 200mg/kg for 1 to 28 days and killed 24 hours after the final dose. Controls received vehicle only. Isolated hepatocytes were examined microscopically for chromosome aberrations and SCEs. Replicative DNA synthesis (RDS) in the hepatocytes was determined by in-vitro 5-bromo-2'-deoxyuridine immunoreactive staining. Polychromatic erythrocytes (PCEs) isolated from the femur were examined for micronuclei. Rats treated with repeated quinoline doses of 200mg/kg exhibited abnormal clinical signs. Body weight gain was significantly lower in rats treated with quinoline doses of 100 and 200mg/kg than in controls. Relative to controls, chromosome aberrations in the rat liver were significantly elevated 4 to 24 hours following administration of 200mg/kg quinoline. SCEs in the rat liver were significantly increased 4 to 48 hours following administration of 200mg/kg quinoline. Significant, dose dependent increases in chromosome aberrations and SCEs were observed following repeated exposure to quinoline doses of 25 to 200mg/kg. RDS in hepatocytes was significantly increased 24 and 48 hours following treatment with 200mg/kg quinoline. Significant, dose dependent increases in RDS were observed in rats exposed repeatedly to quinoline doses of 25 to 200mg/kg. Quinoline failed to induce micronuclei in PCEs. Treatment with 500mg/kg 8HQ caused a significant reduction in body weight gain. Chromosome aberrations, RDS, and micronuclei formation were not influenced by 8HQ. Exposure to 8HQ caused a significant increase in SCE frequency. The authors conclude that quinoline is a genotoxic carcinogen. The above cytogenetic assay can be used as an effective method for determining in-vivo carcinogenicity.

Asano K, Phan L, Anderson J, Hinnebusch AG. **Complex formation by all five homologues of mammalian translation initiation factor 3 subunits from yeast Saccharomyces cerevisiae.** J Biol Chem 1998;273 (29):18573-85.
The PRT1, TIF34, GCD10, and SUI1 proteins of Saccharomyces cerevisiae were found previously to copurify with eukaryotic translation initiation factor 3 (eIF3) activity. Although TIF32, NIP1, and TIF35 are homologous to subunits of human eIF3, they were not known to be components of the yeast factor. We detected interactions between PRT1, TIF34, and TIF35 by the yeast two-hybrid assay and in vitro binding assays. Discrete segments (70-150 amino acids) of PRT1 and TIF35 were found to be responsible for their binding to TIF34. Temperature-sensitive mutations mapping in WD-repeat domains of TIF34 were isolated that decreased binding between TIF34 and TIF35 in vitro. The lethal effect of these mutations was suppressed by increasing TIF35 gene dosage, suggesting that the TIF34-TIF35 interaction is important for TIF34 function in translation. Pairwise in vitro interactions were also detected between PRT1 and TIF32, TIF32 and NIP1, and NIP1 and SUI1. Furthermore, PRT1, NIP1, TIF34, TIF35,

and a polypeptide with the size of TIF32 were specifically coimmunoprecipitated from the ribosomal salt wash fraction. We propose that all five yeast proteins homologous to human eIF3 subunits are components of a stable heteromeric complex in vivo and may comprise the conserved core of yeast eIF3.

Asano N, Katsuma Y, Tamura H, Higashikuni N, Hayashi M. **An automated new technique for scoring the rodent micronucleus assay: computerized image analysis of acridine orange supravitally stained peripheral blood cells.** Mutat Res 1998;404(1-2):149-54.
We developed an automated image analysis system to obtain objective data for the rodent peripheral blood micronucleus assay with acridine orange (AO) supravital staining. The system was able to identify micronucleated reticulocytes (MNRETs) and to evaluate inhibition of bone marrow cell proliferation by measuring the reticular area of reticulocytes (RETs). We also developed automated equipment to produce homogeneous acridine orange-coated glass slides. This study was designed to compare automated scoring with manual scoring using 4 model clastogens and 2 mouse strains. The MNRET incidence induced by each clastogen was similar for automated and manual scoring, and there was good correlation (r=0.92) between the methods. In addition, an index of bone marrow toxicity based on the reticular area of RETs was compared to the conventional index (% of polychromatic erythrocytes (PCE) to total erythrocytes; PCE ratio) and was similar. The results indicated that our technique for computer-assisted image analysis for the micronucleus assay with AO supravitally stained peripheral blood RETs was comparable to conventional microscopic scoring, and it was superior in objectivity and statistical power. Copyright 1998 Elsevier Science B.V.

Assad M, Lemieux N, Rivard CH. **Immunogold electron microscopy in situ end-labeling (EM-ISEL): assay for biomaterial DNA damage detection.** Biomed Mater 1997;7(6):391-400.
We have evaluated a genotoxicity assay that combines in situ end-labeling, colloidal gold tagging and electron microscopy in order to adapt it to the measurement of in vitro biomaterial-induced genotoxicity. Human lymphocytes were cultured in semi-physiological medium which had been previously exposed to biomaterial extracts of commercially pure titanium following ISO standards. In order to visualize the location of induced DNA strand breaks, cells were then exposed to exonuclease III which partially digests and amplifies lesions by releasing nucleotides at free 3' hydroxyl ends from nicked double-stranded DNA. The resulting single-stranded DNA was allowed to hybridize with short oligonucleotides of random sequences including biotinylated dUTP. After random priming using Escherichia coli DNA polymerase I, incorporation of biotin-dUTP was detected by immunogold binding to the chromatin. Cells exposed to a mutagenic concentration of methyl methanesulfonate, as a positive control, showed a significantly higher and stronger gold staining than both titanium-exposed and unexposed specimens. This assay allows a precise localization and quantification of both in vitro DNA breakage and DNA repair. It could provide a powerful tool for rapid assessment of the genotoxic potential of new biomaterials.

Assad M, Yahia LH, Rivard CH, Lemieux N. **In vitro biocompatibility assessment of a nickel-titanium alloy using electron microscopy in situ end-labeling (EM-ISEL).** J Biomed Mater Res 1998;41(1):154-61.
Shape memory nickel-titanium (NiTi) alloys are potential candidates for biomedical applications. However, their equiatomic composition (50 wt% Ni) is controversial, and concerns have been raised about their biocompatibility level because of the carcinogenicity potential. The relative in vitro genotoxicity of NiTi therefore was evaluated and compared to commercially pure titanium (cpTi), 316L stainless steel (SS 316L), and positive and negative controls. To do so, human peripheral blood lymphocytes were cultured in semiphysiological medium that previously had been exposed to the biomaterials. The electron microscopy in situ end-labeling (EM-ISEL) assay then was performed in order to provide quantification of in vitro chromatin DNA single-stranded breaks (SSBs). Chromosomes and nuclei were harvested and exposed to exonuclease III, which amplifies DNA lesions at 3' ends of breaks. After random priming, incorporation of biotin-dUTP was labeled by immunogold binding, which then was detected using electron microscopy. Cellular chromatin exposed to the positive control demonstrated a significantly stronger immunogold labeling than when it was exposed to NiTi, cpTi, SS 316L extracts, or the untreated control. Moreover, gold particle counts, whether in the presence of NiTi, cpTi, or the negative control medium, were not statistically different. NiTi genocompatibility therefore presents promising prescreening results towards its biocompatibility approval.

Awara WM, El-Nabi SH, El-Gohary M. **Assessment of vinyl chloride-induced DNA damage in lymphocytes of plastic industry workers using a single-cell gel electrophoresis technique.** Toxicology 1998;128(1):9-16.
BIOSIS COPYRIGHT: BIOL ABS. DNA damage and the formation of stable carcinogen-DNA adducts are considered critical events in the initiation of the carcinogenic process. This study was carried out to assess whether exposure of plastics industry workers to the vinyl chloride monomer (VCM) for different periods of time would cause DNA damage, using the single-cell gel electrophoresis (SCGE) technique. Levels of DNA damage was assessed by both extent of DNA migration and numbers of DNA damaged spots in the peripheral blood lymphocytes from 32 plastics workers with different periods of exposure to VCM; they were evaluated by comparison with a group of non-exposed individuals. It was found that plastics workers who were exposed to VCM for different periods of time showed significantly increased levels of DNA damage compared with the non-exposed subjects. There was a significant correlation between the severity of DNA damage and duration of exposure. However, no significant correlation was found between the age of all subjects and DNA damage. Concentrations of VCM in the air inside the factory were found to be significantly higher than values in non-exposed areas, despite being lower than the threshold limit value (TLV). Our results encourage the application of SCGE as a sensitive, simple, fast and useful technique in the regular health screening of workers occupationally exposed to VCM (even at concentrations below the TLV) to assess the possibility of any DNA damage.

Badr FM, El-Habit OH, Hamdy M, Hassan GA. **The mutagenic versus protective role of vitamin A on the induction of chromosomal aberration in human lymphocyte cultures.** Mutat Res 1998;414(1-3):157-63.
The present study was carried out to evaluate the role of vitamin A (VA) on the induction of chromosomal aberrations (CA) in lymphocyte culture system and to investigate its modulating effect on chromosomal damage induced by gamma irradiation. Lymphocyte cultures from five healthy normal adult males were either treated with VA at a dose level of 2.0, 8.0 or 24.0 microg/ml or exposed to gamma-irradiation of 3.0 Gy, then followed immediately by a treatment with one of the above mentioned doses of VA. Non-treated cultures and cultures exposed to gamma-irradiation served as control for the two sets of experiments. Cultures were set up in duplicates and incubated for 48 h for assessment of CA. Treatment with VA alone increased CA demonstrating a dose-response effect. Addition of VA to gamma-irradiated cultures resulted in an inverse protective effect as the low dose of 2 &mgr;g/ml reduced the CA induced by radiation to about 1/3 rd whereas a dose of 8 microg/ml had a protective effect of 40% of the total damage and the large dose of 24 microg/ml had no or little effect. These results suggest that a proportion of the added VA may interfere with the radiation induced free radicals and other reactive metabolites which elevate CA. On the other hand, excessive amounts of VA increased toxicity and reduced effect on repair enzymes. Copyright 1998 Elsevier Science B.V.

Banath JP, Fushiki M, Olive PL. **Rejoining of DNA single- and double-strand breaks in human white blood cells exposed to ionizing radiation.** Int J Radiat Biol 1998;73(6):649-60.
PURPOSE: To characterize inter- and intra-individual differences in X-ray-induced DNA strand break rejoining kinetics in human peripheral white blood cells (WBC) obtained from 10 healthy volunteers. MATERIALS AND METHODS: The alkaline and neutral versions of the comet assay were used to measure the rate of rejoining of predominantly single-strand breaks (ssb) following exposure to 8 Gy and double-strand breaks (dsb) following 75 Gy. RESULTS: All cells within a population responded in a similar fashion to induction of ssb and dsb; however, a subset of the WBC appeared to rejoin ssb more rapidly. For the 10 individuals examined, the percentage of ssb rejoined by the rapid component(s) was 47 +/- 16% and the rejoining half-time for the slow component was 1.3 +/- 0.4 h. By 24 h after 8 Gy, 4.9 +/- 3.8% of the initial ssb remained. For dsb rejoining, 58 +/- 11% of the initial damage was still present 4h after 75 Gy and by 24 h 32% of the initial level of damage was still detected. Heavily damaged cells present 24 h after 75 Gy varied from 4% to 50% and were excluded from the analysis of repair rates. CONCLUSIONS: Inter-individual variability exceeded intra-individual variability for 2 of 4 endpoints examined for ssb repair, but not for dsb repair. It was concluded that DNA damage measured using the comet assay could identify a range in the X-ray repair responses of WBC from different normal individuals. Whether these differences correlate with differences in cell killing by radiation remains to be determined.

Beamand JA, Barton PT, Price RJ, Lake BG. **Lack of effect of coumarin on unscheduled DNA synthesis in**

precision-cut human liver slices. Food Chem Toxicol 1998;36(8):647-53.
In this study the effect of coumarin on unscheduled DNA synthesis (UDS) in precision-cut human liver slices has been examined. Liver slices from tissue samples from four donors were cultured for 24 hr in medium containing [3H] thymidine and 0-5.0 mM coumarin using a dynamic organ culture system and processed for autoradiographic evaluation of UDS. As positive controls liver slices were also cultured with three known genotoxic agents, namely 0.02 and 0.05 mM 2-acetylaminofluorene (2-AAF), 0.002 and 0.02 mM aflatoxin B1 (AFB1) and 0.005 and 0.05 mM 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP). UDS was quantified as the net grain count in centrilobular hepatocytes and as the percentage of centrilobular hepatocyte nuclei with more than five net grains. Compared with control liver slice cultures, treatment with 0.05-5.0 mM coumarin had no effect on UDS. In contrast, treatment with 0.02 and 0.05 mM 2-AAF, 0.002 and 0.02 mM AFB1 and 0.005 and 0.05 mM PhIP produced significant increases in the net grain counts of centrilobular hepatocytes. The greatest induction of UDS was observed in liver slices treated with 0.05 mM PhIP. Treatment with 2-AAF, AFB1 and PhIP also produced significant increases in the number of centrilobular hepatocyte nuclei with more than five net grains. At the concentrations examined neither coumarin. 2-AAF, AFB1 nor PhIP had any significant effect on replicative DNA synthesis in 24 hr cultured human liver slices. These results demonstrate that coumarin does not induce UDS in cultured human liver slices. However, all three positive control compounds produced marked significant increases in UDS, thus confirming the functional viability of the human liver slice preparations used in this study. The results of this study suggest that coumarin is not a genotoxic agent in human liver.

Beamand JA, Barton PT, Tredger JM, Price RJ, Lake BG. **Effect of some cooked food mutagens on unscheduled DNA synthesis in cultured precision-cut rat, mouse and human liver slices.** Food Chem Toxicol 1998;36(6):455-66.
Precision-cut liver slices were prepared from male Fischer 344 rats, female CDF1 mice and humans (both male and female subjects). Liver slices were cultured for 24 hr in medium containing [3H]thymidine and either PhIP, IQ, MeIQ, MeIQx, Glu-P-1 or Trp-P-1, and then processed for auto-radiographic evaluation of unscheduled DNA synthesis (UDS). All six cooked food mutagens examined produced concentration-dependent increases in UDS in human liver slices. PhIP was the most potent compound examined, followed by MeIQx, IQ and then MeIQ, Glu-P-1 and Trp-P-1. Significant increases in UDS were observed with PhIP, IQ and MeIQx at concentrations as low as 5 microM in the culture medium. The same rank order of potency was not apparent in either rat or mouse liver slices. In rat liver slices only MeIQ significantly induced UDS, although positive results were obtained with two other genotoxins, namely 2-acetylaminofluorene and aflatoxin B1. Apart from MeIQx, all the cooked food mutagens produced significant increases in UDS in mouse liver slices. This study demonstrates the usefulness of precision-cut liver slices to evaluate species differences in xenobiotic-induced genotoxicity. Both marked compound and species differences in induction of UDS were observed. The data provide further evidence that dietary cooked food mutagens are potential human carcinogens.

Belpaeme K, Cooreman K, Kirsch-Volders M. **Development and validation of the in vivo alkaline comet assay for detecting genomic damage in marine flatfish.** Mutat Res 1998;415(3):167-84.
Biomonitoring is an important subject within environmental sciences. Biomonitoring tests are required to be quick, relatively inexpensive, accurate, and reproducible. No genetic test currently fulfils all of these requirements. The chromosome aberration and sister chromatid exchange tests are very time consuming, the DNA adduct technique is rather expensive, and the micronucleus test has not inconclusively proven its use as a reliable monitoring tool. This work is focused on the validation of the comet assay as a candidate for monitoring marine ecosystems. For the comet assay, this work deals with the effectiveness of tissue dissociation, storage of cells in lysing buffer and in liquid nitrogen, different electrophoretic conditions, neutralisation and fixation of slides, interindividual variation between samples, and responsiveness of four tissue types to ethyl methanesulphonate (EMS). The main conclusions are: (i) dissociation of solid tissues in a phosphate buffer supplemented with 200 mM N-t-butyl-alpha-phenylnitrone provides cells with an acceptable background DNA damage; (ii) freezing of cells or tissues in liquid nitrogen generally leads to an increase in DNA breakage, especially for liver, gill and kidney tissue; (iii) storage of slides in the lysing solution for up to one week gives minor changes in comet tails; (iv) differences in protocols for neutralisation and fixation may influence the results; (v) high intra- and interindividual variations in comets (length and DNA content) may obscure the interpretation of comet results; (vi) blood, gill, liver and kidney all showed a

statistically significant increase of DNA damage after exposure to 50 mg EMS/l; (vii) electrophoresis at low voltage for longer periods is to be preferred to high voltage and short electrophoresis times. The simplicity and sensitivity of the comet assay make it an adequate test system for biomonitoring of chronic low level exposure. However, protocols and experimental conditions have to be chosen carefully.

Benigni R, Passerini L, Gallo G, Giorgi F, Cotta-Ramusino M. **QSAR models for discriminating between mutagenic and nonmutagenic aromatic and heteroaromatic amines.** Environ Mol Mutagen 1998;32(1):75-83.
In a previous article, we demonstrated that the structure-activity relationship model for the mutagenic potency of aromatic amines is different from that for discriminating between mutagens and nonmutagens. In this work, we present further analyses on the molecular determinants of the mutagenicity of aromatic amines. Based on the use of various methodological approaches, our results indicate that mutagenic activity is influenced by different molecular characteristics in different subclasses of aromatic amines. Thus, the general lesson of this article is that 1) in genetic toxicology, it is necessary to separately investigate the structure-activity relationships for discrimination between positive and negative chemicals, and the structure-activity relationships for the potency of the positive chemicals; 2) in structure-activity studies, it is necessary to investigate the degree of homogeneity (congenericity) of apparently similar chemicals in order to assess and describe the various mechanisms of action that may be elicited by the chemicals.

Berthe-Corti L, Jacobi H, Kleihauer S, Witte I. **Cytotoxicity and mutagenicity of a 2,4,6-trinitrotoluene (TNT) and hexogen contaminated soil in S. typhimurium and mammalian cells.** Chemosphere 1998;37(2):209-18.
The toxicity and mutagenicity of aqueous and organic extracts of soil contaminated with TNT, TNT metabolites and hexogen was determined in mammalian cell lines and in prokaryotic cells. The prokaryotic toxicity was determined via the colony forming ability of Salmonella typhimurium (strains TA 98 and TA 100). The same strains were used to test mutagenicity in the Ames test. The mammalian toxicity was analyzed in human fibroblasts by the inhibition of cell growth and cell viability (MTT assay). The mammalian mutagenicity was tested with the HPRT test in V79 cells (hamster lung). The aqueous soil extract did not reveal toxicity or mutagenicity in any of the tests performed. The DMSO/ethanol extract showed toxicity and mutagenicity in S. typhimurium. Thereby strain TA 98 was more sensitive than strain TA 100. In human fibroblasts cell growth was strongly inhibited, whereas no reduction of cell viability was found in the MTT test. Mutagenicity of the DMSO/ethanol extract of the soil was demonstrated in V79 cells.

Best CJ, McKenna PG, McKelvey-Martin VJ. **Mutagen sensitivity in thymidine kinase- and methyltransferase-deficient human lymphoblastoid cells.** Br J Biomed Sci 1997;54(4):267-72.
In this study, the effect of thymidine kinase deficiency on the responses of the human lymphoblastoid cell line Raji to methyl methanesulphonate and mitomycin C was investigated. Mutagen sensitivity was measured in terms of cell survival and mutation to hypoxanthine-guanine phosphoribosyltransferase deficiency. Thymidine kinase-deficient Raji cells showed decreased survival and increased mutant frequency relative to wild-type cells following treatments with each of the mutagens used. It is suggested that this may be due to an imbalance in the supply of deoxyribonucleoside triphosphates to the excision repair process. The role of O6-methylguanine-DNA methyltransferase in the repair of DNA damage caused by these mutagens is also discussed.

Bhanoori M, Venkateswerlu G. **The alkaline single cell gel electrophoresis: a new test for assessing DNA single strand breaks in Neurospora crassa.** Mutat Res 1998;405(1):29-34.
The single cell gel electrophoresis (comet assay) is a potent technique in testing double and single strand breaks in DNA. In this paper, we present an application of alkaline comet assay to filamentous fungi for genotoxicological assessment of heavy metals for the first time. A wild strain of Neurospora crassa SLA 4200 was grown in presence of cadmium sulfate (CdSO4) (10 microM and 100 microM) for 12 h. Protoplasts from 12-h old mycelia were prepared by using Novozym 234 and DNA damage was evaluated by alkaline comet assay. Hydrogen peroxide (H2O2) (50 microM and 100 microM) was taken as an internal standard for DNA damage. Both CdSO4 and H2O2 induced significant single strand breaks in DNA. The results indicate that alkaline comet assay is a sensitive and rapid method for DNA damage analysis in filamentous fungal systems. Copyright 1998 Elsevier Science B.V.

Bhattacharjee R, Saxena M, Tyagi BR, Singh H, Singh V, Kumar S. **Mutagenic effectiveness and efficiency of gamma-rays, ethyl methane-sulphonate and nitroso-methyl urea in periwinkle (Catharanthus roseus).** J Nucl Agric Biol 1998;27(1):61-4.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE CATHARANTHUS-ROSEUS PERIWINKLE MUTAGENIC EFFECTIVENESS MUTAGENIC EFFICIENCY GAMMA-RAY ETHYL METHANE-SULFONATE NITROSO-METHYL UREA HORTICULTURE RADIATION BIOLOGY MUTAGEN.

Bhattacharya R, Rao PVL. **Cyanide induced DNA fragmentation in mammalian cell cultures.** Toxicology 1997;123(3):207-15.
The genotoxic potential of cyanide (57125) was studied in mammalian cell cultures. Thymocytes freshly isolated from male Wistar-rats and commercial baby hamster kidney BHK-21 cell preparations were incubated with 0 to 10 millimolar (mM) potassium-cyanide (151508) for 1 to 24 hours in medium containing 1mM calcium-chloride (10043524) or in calcium (Ca2+) free medium. Some cells were also simultaneously treated with 1 to 2.5mM divalent zinc (7440666) (Zn2+), a modulator of Ca2+/magnesium dependent endonuclease, from zinc-chloride (7646857), 2.5mM N-acetylcysteine (616911) (NAC), a free radical scavenger, or 2.5mM diltiazem (42399417), a Ca2+ channel blocker. Cytotoxicity was assessed by measuring leakage of intracellular lactate-dehydrogenase (LDH) into the medium and by the eosin-Y dye exclusion test. Genotoxicity was assessed by measuring the extent of DNA strand breaks by a fluorimetric procedure and by measuring the extent of DNA fragmentation in whole cells (internucleosomal DNA fragmentation (IDF)) by a gel electrophoresis assay. In rat thymocytes, cyanide induced dose and time dependent cytotoxicity and DNA strand breakage. Cyanide at doses of 2.5mM or higher induced IDF. DNA damage and cytotoxicity induced by 5mM cyanide was attenuated by 2.5mM Zn2+, NAC, and diltiazem except for LDH leakage. Zn2+ showed the strongest protective effect. The extent of DNA damage was minimal when thymocytes were exposed to cyanide in Ca2+ free medium. In BHK-2 cells, cyanide at doses of 5 and 10mM was cytotoxic and induced DNA strand breakage. Cyanide did not induce IDF. The authors conclude that cyanide causes dose and time dependent IDF in rat thymocytes. The DNA damage may reflect endonucleolytic DNA degradation associated with cytotoxicity rather than a direct interaction with DNA. The DNA damage depends on the presence of extracellular Ca2+ and is ameliorated by Zn2+, NAC, and diltiazem. BHK-21 cells are relatively resistant to the effects of cyanide, as indicated by no IDF being observed.

Blanco M, Urios A, Martinez A. **New Escherichia coli WP2 tester strains highly sensitive to reversion by oxidative mutagens.** Mutat Res 1998;413(2):95-101.
New Escherichia coli strains have been added to the WP2 mutagenicity test for the specific detection of oxidative mutagens. Strain IC203 derives from WP2 uvrA/pKM101 and is highly sensitive to oxidative stress due to a deficiency in the OxyR function. Following exposure to t-butyl hydroperoxide (BuOOH) or menadione (MD), but not to 4-nitroquinoline 1-oxide (4NQO), strain IC203 (oxyR) shows increased mutability with respect to the oxyR+ parent. The advantage that the OxyR deficiency confers on IC203 strain in detecting oxidative mutagens is not obtained with strains deficient in either katG or ahpCF, two OxyR-regulated genes. Strain IC206, a derivative of WP2 uvrA carrying a deletion of the umuDC genes and deficient in the MutY glycosylase, has also been added to the WP2 test for the detection of SOS-independent mutations promoted by 8-oxoguanine lesions. Induction of these mutations was observed after treatment with BuOOH, but not after MD or 4NQO exposure. The two new strains, IC203 and IC206, can be useful for the screening of mutations resulting from oxidative stress as well as in studies on antioxidants preventing mutagenesis. Copyright 1998 Elsevier Science B.V.

Buchholz F, Angrand PO, Stewart AF. **Improved properties of FLP recombinase evolved by cycling mutagenesis [see comments].** Nat Biotechnol 1998;16(7):657-62.
The site-specific recombinases FLP and Cre are useful for genomic engineering in many living systems. Manipulation of their enzymatic properties offers a means to improve their applicability in different host organisms. We chose to manipulate the thermolability of FLP recombinase. A lacZ-based recombination assay in Escherichia coli was used for selection in a protein evolution strategy that relied on error-prone PCR and DNA shuffling.

Improved FLP recombinases were identified through cycles of increasing stringency imposed by both raising temperature and reducing protein expression, combined with repetitive cycles of screening at the same stringency to enrich for clones with improved fitness. An eighth generation clone (termed FLPe) showed improved properties in E. coli, in vitro, in human 293- and mouse ES-cells.

Bunger J, Krahl J, Franke HU, Munack A, Hallier E. **Mutagenic and cytotoxic effects of exhaust particulate matter of biodiesel compared to fossil diesel fuel.** Mutat Res 1998;415(1-2):13-23.
The mutagenic and cytotoxic effects of diesel engine exhaust (DEE) from a modern passenger car using rapeseed oil methyl esters (RME, biodiesel) as fuel were directly compared to DEE of diesel fuel (DF) derived from petroleum. Combustion particulate matter was collected on glass fiber filters coated with polytetrafluoroethylene (PTFE) from an exhaust dilution tunnel using three different engine test cycles on a chassis dynamometer. Filters were extracted with dichloromethane in a soxhlet apparatus for 12 h. The mutagenicity of the extracts was tested in the Salmonella typhimurium/mammalian microsome plate-incorporation assay using strains TA97a, TA98, TA100, and TA102. The toxicity to the established cell line L929 (mouse lung fibroblasts) was investigated in the neutral red assay. In the tester strains TA98 and TA100 a significant increase of mutations resulted for the particle extracts of both fuels, but for DF the revertants were significantly higher compared to RME. The highest levels of revertants were observed in tests including a cold start phase. This was probably due to incomplete combustion in the cold engine and a lower conversion rate of the cold catalytic converter. Testing with activated liver S9 fraction induced a slightly lower increase of revertants in most experiments. TA97a and TA102 showed no significant enhancement of spontaneous mutations. In the FTP-75 test cycle RME extracts showed slightly higher toxic effects to the L929 cells than DF, whereas in the other tests no significant differences were observable. These results indicate a higher mutagenic potency of DEE of DF compared to RME. This is probably due to the lower content of polycyclic aromatic compounds (PAC) in RME exhaust, although the emitted masses of RME were higher in most test procedures applied in this study.

Burcham PC. **Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts.** Mutagenesis 1998;13(3):287-305.
BIOSIS COPYRIGHT: BIOL ABS. The peroxidation of polyunsaturated lipids generates a range of substances that possess DNA damaging potential. This includes lipid hydroperoxides and various species that contain unpaired electrons, such as the alkoxyl and peroxyl radicals. In addition, a range of genotoxic carbonyl-containing compounds are formed, such as malondialdehyde, various 4-hydroxy-2-alkenals such as 4-hydroxynonenal and a number of 2-alkenals. It has previously been assumed that the antioxidants and electrophile scavenging enzymes existing in mammalian cells effectively protect the genetic material against these substances. However, thanks to recent analytical advances in the detection of low levels of DNA adducts, it is now evident that DNA adducts formed from a range of lipid peroxidation products are abundant in both rodent and human genomes. This suggests that the cellular defence system is not 100% efficient and that a proportion of endogenously produced lipid peroxidation products escape detoxification and cause DNA damage. This review surveys the genotoxic properties of the major classes of lipid peroxidation products, focusing on their chemistry of DNA adduction, the mutagenic properties of such damage and the evidence that it occurs in intact biological systems. Furthermore, avenues of future research that will clarify the significance of such damage to spontaneous mutagenesis and carcinogenesis are proposed and discussed.

Buzaid AC, Ali-Osman F, Akande N, Grimm EA, Lee JJ, Bedikian A, Eton O, Papadopoulos N, Plager C, Legha SS, et al. **DNA damage in peripheral blood mononuclear cells correlates with response to biochemotherapy in melanoma.** Melanoma Res 1998;8(2):145-8.
The combination of cisplatin-based chemotherapy with interleukin-2 (IL-2) and interferon, referred to as biochemotherapy, has shown encouraging results in patients with advanced melanoma. Toxicity is high, however and no objective parameters exist to distinguish between patients who are likely to respond and those who are not. The purpose of this pilot study was to determine whether in vitro cisplatin-induced damage to the glutathione S-transferase-pi (GST-pi) gene in peripheral blood mononuclear cells (PBMCs) before therapy correlated with the histological response in melanoma patients with local-regional metastases who received concurrent

biochemotherapy before definitive surgery. Before therapy, PBMCs from 16 patients were exposed to cisplatin at concentrations of 25, 50 or 100 microM for 3 h and the extent of damage to the GST-pi gene was quantitated by polymerase chain reaction (PCR). Patients were subsequently treated on a biochemotherapy regimen consisting of cisplatin 20 mg/m2 intravenously (i.v.) on days 1-4, vinblastine 1.5 mg/m2 i.v. on days 1-4, dacarbazine 800 mg/m2 i.v. on day 1, IL-2 9 MIU/m2 per day i.v. by continuous infusion on days 1-4 (total of 96 h), and interferon alpha2a 5 MU/m2 subcutaneously on days 1-5. The 16 patients were categorized into two groups: major responders (n = 7) and non-major responders (n = 9). Although we observed a wide interpatient variation, a statistically significant correlation existed between the histological response and the degree of DNA damage caused in the PBMCs at all three cisplatin concentrations tested (P = 0.024 for 25 microM; P = 0.036 for 50 microM; P = 0.007 for 100 microM). Our pilot study suggests that determination of in vitro cisplatin-induced DNA damage using a gene-specific PCR assay may be useful in predicting the histological response to biochemotherapy.

Cariello NF, Narayanan S, Kwanyuen P, Muth H, Casey WM. **A novel bacterial reversion and forward mutation assay based on green fluorescent protein.** Mutat Res 1998;414(1-3):95-105.
BIOSIS COPYRIGHT: BIOL ABS. We report the first use of green fluorescent protein (GFP) for mutation detection. We have constructed a plasmid-based bacterial system whereby mutated cells fluoresce and non-mutated cells do not fluoresce. Fluorescence is monitored using a simple hand-help UV lamp; no additional cofactors or manipulations are necessary. To develop a reversion system, we introduced a +1 DNA frameshift mutation in the coding region of GFP and the resulting protein is not fluorescent in Escherichia coli. Treatment of bacteria containing the +1 frameshift vector with ICR-191 yields fluorescent colonies, indicating that reversion to the wild-type sequence has occurred. Site-directed mutagenesis was used to insert an additional cytosine into a native CCC sequence in the coding region of GFP in plasmid pBAD-GFPuv, expanding the sequence to CCCC. A dose-related increase in fluorescent colonies was observed when the bacteria were treated with ICR-191, an agent that induces primarily frameshift mutations. The highest dose of ICR-191 tested, 16 mug/ml, produced a mutant fraction of 16plicate experiments. The reversion system did not respond to MNNG, an agent that produces mainly single-base substitutions. To develop a forward system, we used GFP under the control of the arabinose PBAD promoter; in the absence of arabinose, GFP expression is repressed and no fluorescent colonies are observed. When cells were treated with MNNG or ENNG, a dose-dependent increase in fluorescent colonies was observed, indicating that mutations had occurred in the arabinose control region that de-repressed the promoter. Treating bacteria with 100 mug/ml MNNG induced mutant fractions as high as 82licate experiments. Treating bacteria with 150 mug/ml ENNG induced a mutant fraction of 2.1.

Carrera P, De Miguel M, Lopez J, De La Torre C, Navarrete MH. **In vivo response of mouse liver to gamma-radiation assessed by the comet assay.** Mutat Res 1998;413(1):23-31.
BIOSIS COPYRIGHT: BIOL ABS. The alkaline comet assay was used to measure DNA damage induced in liver cells of mice irradiated with gamma-radiation. as well as the repair competency of these cells. A simplified procedure for the isolation of nuclei from cells in solid tissues was developed. This simplified method allows nuclei to be processed into lysis only 5 min after briefly chilling the tissue to depress any enzymatic activity. The nuclei were spontaneously released by a sharp cut of the tissue and exposure of the cut t a drop of 50 mM sodium-phosphate buffer at pH 7.2, immediately before adding the low melting agarose. Thus, the procedure minimizes time-dependent modification of the endogenous level of damage by reducing additional strand breaks or repair produced during processing. The induction of DNA damage by gamma-radiation behaved as a one-hit event in the liver cells, as there was a positive linear correlation between the radiation dose and the fraction of DNA migrated into the comet tails. The level of DNA damage produced by gamma-radiation was highly significant at doses of 0.5 and 1 Gy. Based on the mean extent of DNA migration, the level of damage was not reduced following only one hour of repair time however, after two hours, there was a significant reduction in DNA migration. To increase the resolution of the statistical analysis, the nuclei of each sample were distributed in five types of comets, according to the percentage of DNA in the tail. To compare the frequency distributions of these types of comets between different experimental situations, a Pearson chi-square statistical analysis was applied. It was found, by this analysis, that the DNA repair which occurred 1 h after 1 Gy of gamma-irradiation is significant and that, after 2 h, more DNA repair occurs, but a significant residual damage still persists when comparing this sample with the control.

Cebulska-Wasilewska A, Nowak D, Niedzwiedz W, Anderson D. **Correlations between DNA and cytogenetic damage induced after chemical treatment and radiation.** Mutat Res 1998;421(1):83-91.
The induction of damage in human lymphocytes has been compared after treatment in vitro with two different agents, the chemical o-phenylenediamine (o-PDA) and gamma irradiation, in the alkaline single cell gel electrophoresis (Comet) assay, and after cytogenetic analysis. The chemical treatment caused dose-related increases in DNA damage in the Comet assay and cytogenetic damage in the first and second metaphases. The results revealed a very strong association between the two types of damage. Correlation coefficients were from 0.95 to 0.97. From previous studies, high correlation coefficients of 0.99 and 0.97 in the same assays were also evaluated for X-rays and fast neutrons, respectively. On the basis of such results, we suggest that the Comet assay responses provide a good prediction of cytogenetic damage. Thus, because of its simplicity and rapidity, the Comet assay would appear to be a very useful tool for determining the genotoxicity of environmental agents. Copyright 1998 Elsevier Science B.V.

Cerna M, Pastorkova A, Myers SR, Rossner P, Binkova B. **The use of a urine mutagenicity assay in the monitoring of environmental exposure to genotoxins.** Mutat Res 1997;391(1-2):99-110.
A study was conducted to evaluate and monitor environmental exposure to genotoxins using two urine mutagenic activity assays, a standard Salmonella plate incorporation assay, and a microsuspension assay modification. Spot urine samples were obtained from 28 women exposed to high air pollution levels in Teplice (TP), in Northern Bohemia, and 24 women exposed to lower air pollution levels in Prachatice (PT), in Southern Bohemia. Urinary polycyclic aromatic hydrocarbons (PAHs) and metabolites were analyzed using high performance liquid chromatography and gas chromatography mass spectrometry. In the plate incorporation assay, the Salmonella-typhimurium strains TA-98 and YG1041 were exposed to various urine concentrations in the presence or absence of S9 fraction. In the microsuspension assay, the YG1041 strain was exposed to various urine concentrations in the presence or absence of S9 fraction. The number of revertants per plate was determined after 72 hours of incubation. In the plate incorporation assay, the number of induced revertants was higher with the YG1041 strain than with the TA-98 strain. The mean number of revertants in the metabolically activated YG1041 cultures was significantly lower in the PT urine than in the TP urine. Although the mean number of induced revertants was higher in the microsuspension assay than in the plate incorporation assay, the microsuspension assay results did not differ between the groups. In both assays, the number of revertants in metabolically activated YG1041 cultures was significantly correlated with that in the unactivated YG1041 cultures. Urinary PAH and metabolite concentrations were significantly higher in the TP group than in the PT group. The excretion of PAHs and metabolites was not affected by smoking status. The number of revertants in metabolically activated YG1041 cultures in the plate incorporation assay was significantly correlated with most of the PAH and metabolite urinary concentrations. The authors conclude that the use of the plate incorporation assay with the YG1041 strain in conjunction with urinary PAH excretion measurement may be suitable for monitoring environmental exposure to genotoxins.

Chlopkiewicz B, Gruber B. **The in vitro study on genotoxic activity of adriamycin and bleomycin in cells of mice with different catalase and superoxide dismutase activity.** Acta Pol Pharm 1997;54(6):437-41.
Genotoxic activity of adriamycin and bleomycin in embryo cells from mice differing in anti-oxidant enzymes activity was investigated. The catalase activity in cultured in vitro embryo cells of C3H mice was 2.3-fold and superoxide dismutase 2.5-fold higher than of C57BL/10 mice. For genotoxicity evaluation, the micronucleus test in vitro was used. The results obtained indicated that the frequency of micronucleated cells in untreated C3H cultures was higher than in C57BL/10 cell cultures. The increase in micronuclei formation after treatment with adriamycin and bleomycin was higher in C57BL/10 than in C3H cells as compared with micronuclei in untreated cultures. The higher frequency of micronucleated cells in treated versus untreated C57BL/10 than C3H cell cultures may be caused by lower activity of anti-oxidant enzymes in C57BL/10 cells. It may suggest that DNA damage caused by adriamycin and bleomycin resulted from action of active oxygen species.

Collins A, Dusinska M, Franklin M, Somorovska M, Petrovska H, Duthie S, Fillion L, Panayiotidis M, Raslova K, Vaughan N. **Comet assay in human biomonitoring studies: reliability, validation, and applications.** Environ

Mol Mutagen 1997;30(2):139-46.
Modifications to single cell gel electrophoresis or comet assay, by including digestion with lesion specific repair endonucleases, were developed to obtain specific information regarding oxidative DNA damage and its repair in humans. Duplicate gels prepared from the same sample of cells were compared and the natural intraindividual and interindividual variability in lymphocyte DNA damage were measured in groups of normal, healthy human volunteers. The assay was applied in investigations of human disease and occupational exposure of factory workers. The authors warn that given the speed and sensitivity of the comet assay, it is relatively easy to amass large amounts of data in a human biomonitoring trial. They note that it is important to be aware of the limitations of the assay and not to over interpret the data. Considerable variation was noted in samples of cells collected from the same person on separate occasions. Even so, differences between groups of individuals can reliably be measured, given only a sufficient number of individuals per group.

Criswell KA, Krishna G, Zielinski D, Urda GA, Theiss JC, Juneau P, Bleavins MR. **Use of acridine orange in flow cytometric assessment of micronuclei induction.** Mutat Res 1998;414(1-3):63-75.
BIOSIS COPYRIGHT: BIOL ABS. The micronucleus assay is a widely accepted method for evaluation of clastogens and aneugens. In the current study, acridine orange (AO) supravital staining was adapted for flow cytometric usage to assess micronucleated cells in rat bone marrow and spleen. Cyclophosphamide was used as a positive control test compound and results were compared to manual scoring in Wright-stained slides. In bone marrow, both manual and flow cytometric methods demonstrated positive dose response-trends for micronucleated polychromatic erythrocytes (MNPCE). Significant elevations in MNPCE were observed at all doses of cyclophosphamide, and comparisons between methods in bone marrow were not statistically different. The flow cytometric method was more sensitive in spleen samples, showing dose- and time-related increases in micronuclei compared with manual scoring. AO proved to be a sensitive discriminator of RNA and DNA, allowing distinct separation of polychromatic erythrocytes (PCE), normochromic erythrocytes (NCE), total nucleated cells (TNC), and micronucleated populations within both PCE and NCE regions. These results support the use of AO-based flow cytometry to provide a rapid and sensitive indicator of micronuclei inducers or non-inducers.

Criswell KA, Krishna G, Zielinski D, Urda GA, Theiss JC, Juneau P, Bleavins MR. **Use of acridine orange in flow cytometric evaluation of erythropoietic cytotoxicity.** Mutat Res 1998;414(1-3):49-61.
BIOSIS COPYRIGHT: BIOL ABS. Cytotoxic insult to bone marrow frequently impairs the proliferating and maturational abilities of erythroid cells. Typically, a ratio of enucleated, immature polychromatic erythrocytes (PCE) to mature normochromatic erythrocytes (NCE) is used to assess cytotoxicity in the micronucleus (MN) assay. The effects of cyclophosphamide (CP) on PCE/NCE ratio in rat bone marrow and spleen were assessed by a newly developed flow cytometric procedure using glutaraldehyde-fixed, acridine orange (AO)-stained cells, and compared to manual scoring of PCE/NCE in Wright stained slides. Comparison of methods showed that manual and flow cytometric determination of PCE were not statistically different. Several other parameters of cytotoxicity could be simultaneously assessed because the method allowed use of unfractionated whole bone marrow/spleen cell samples. Absolute numbers of total nucleated cells (TNC), a ratio of TNC to total erythrocytes (TE), and determination of RNA content within the PCE population demonstrated dose- and time-dependent effects with CP treatment. Shifts in RNA content were particularly sensitive, correctly identifying all CP-treated from control specimens, even in those samples where PCE/NCE ratio was similar. The AO methodology provided a more rapid, statistically-superior, and thorough approach in the assessment of bone marrow and spleen cytotoxicity than the conventional manual method of scoring PCE/NCE ratio alone.

Drost JB, Lee WR. **The developmental basis for germline mosaicism in mouse and Drosophila melanogaster.** Genetica 1998;102-103(1-6):421-43.
Data involving germline mosaics in Drosophila melanogaster and mouse are reconciled with developmental observations. Mutations that become fixed in the early embryo before separation of soma from the germline may, by the sampling process of development, continue as part of germline and/or differentiate into any somatic tissue. The cuticle of adult D. melanogaster, because of segmental development, can be used to estimate the proportion of mutant nuclei in the early embryo, but most somatic tissues and the germlines of both species continue from

samples too small to be representative of the early embryo. Because of the small sample of cells/nuclei that remain in the germline after separation of soma in both species, mosaic germlines have percentages of mutant cells that vary widely, with a mean of 50% and an unusual platykurtic, flat-topped distribution. While the sampling process leads to similar statistical results for both species, their patterns of development are very different. In D. melanogaster the first differentiation is the separation of soma from germline with the germline continuing from a sample of only two to four nuclei, whereas the adult cuticle is a representative sample of cleavage nuclei. The presence of mosaicism in D. melanogaster germline is independent of mosaicism in the eye, head, and thorax. This independence was used to determine that mutations can occur at any of the early embryonic cell divisions and still average 50% mutant germ cells when the germline is mosaic; however, the later the mutation occurs, the higher the proportion of completely nonmutant germlines. In contrast to D. melanogaster, the first differentiation in the mouse does not separate soma from germline but produces the inner cell mass that is representative of the cleavage nuclei. Following formation of the primitive streak, the primordial germ cells develop at the base of the allantois and among a clonally related sample of cells, providing the same statistical distribution in the mouse germlines as in D. melanogaster. The proportion of mutations that are fixed during early embryonic development is greatly underestimated. For example, a DNA lesion in a postmeiotic gamete that becomes fixed as a dominant mutation during early embryonic development of the F1 may produce an individual completely mutant in the germ line and relevant somatic tissue or, alternatively, the F1 germline may be completely mutant but with no relevant somatic tissues for detecting the mutation until the F2. In both cases the mutation would be classified as complete in the F1 and F2, respectively, and not recognized as embryonic in origin. Because germ cells differentiate later in mammalian development, there are more opportunities for correlation between germline and soma in the mammal than Drosophila. However, because the germ cells and any somatic tissue, like blood, are derived from small samples, there may be many individuals that test negative in blood but have germlines that are either mosaic or entirely mutant.

Dusinska M, Kovacikova Z, Vallova B, Collins A. **Responses of alveolar macrophages and epithelial type II cells to oxidative DNA damage caused by paraquat.** Carcinogenesis 1998;19(5):809-12.
Because lung cells are inevitably exposed to chemicals, drugs and mineral particles, they are appropriate target cells for investigating effects of environmental toxins. We have studied alveolar macrophages and epithelial type II pneumocytes freshly isolated from the rat lung, using the comet assay to detect DNA damage (strand breaks and oxidized bases) in individual cells after treatment with the pesticide paraquat. The background level of strand breaks is five times higher in freshly isolated pneumocytes than in alveolar macrophages. This difference remains even after 48 h of in vitro culture and therefore probably does not reflect trauma suffered during isolation. In contrast, endogenous formamidopyrimidine glycosylase- and endonuclease III-sensitive sites, which are specific indicators of oxidative damage, are present in freshly isolated alveolar macrophages but not in pneumocytes, reflecting the high metabolic activity of macrophages and their defensive role. Both cell types are exquisitely sensitive to strand breakage by paraquat. In addition, specific base oxidation is detected after 24 h of treatment with paraquat, especially in alveolar macrophages. Susceptibility to DNA damage, rather than lipid peroxidation, is likely to be the cause of paraquat-induced death in these cells. The relatively high level of endogenous damage in pneumocytes suggests that these cells are inefficient at DNA repair, which would be consistent with their probable role as the principal progenitors of lung cancer.

Edenharder R, Frangart J, Hager M, Hofmann P, Rauscher R. **Protective effects of fruits and vegetables against in vivo clastogenicity of cyclosphosphamide or benzo[a]pyrene in mice.** Food Chem Toxicol 1998;36 (8):637-45.
Seven fruits and 10 vegetables commonly consumed in Germany were investigated for their anticlastogenic potencies against cyclophosphamide (CP) and benzo[a]pyrene (BaP) in the in vivo mouse bone marrow micronucleus assay. We detected protective effects in 76.5% and 70.6% of the samples, respectively, and more or less distinct quantitative differences between the various plant materials and the two clastogens investigated. With respect to CP, moderate activities were exerted by sweet cherries, strawberries, cucumber, radish and tomatoes, average activities by bananas, oranges, peaches, asparagus and red beets and strong activities by yellow red peppers and especially spinach. Apples (cultivar Jona Gold), brussels sprouts, cauliflower and onions were inactive.

With respect to BaP, we found moderate activities in strawberries, brussels sprouts and radish, average activities in sweet cherries, oranges, peaches, asparagus, red beets, cucumber and spinach and strong activities in bananas and kiwi. Apples, cauliflower, onions, tomatoes and yellow-red peppers were inactive. When oranges were fractionated according to previously described schemes (Edenharder et al., 1995), anticlastogenic activities against CP were exerted by materials extracted with n-hexane, acetone and 2-propanol and in the terminal residue, but not in the dichloromethane and water phases. With respect to BaP, materials extracted with acetone showed strong anticlastogenicity while the 2-propanol fraction, the aqueous phase and the terminal residue were less potent. The n-hexane and the dichloromethane fractions were inactive. In red beets, all fractions showed anticlastogenicity against CP and BaP as well. However, the n-hexane and dichloromethane fractions were most potent with respect to CP, while for BaP the aqueous phase and the terminal residue were most effective. These result suggest the presence of various (groups of) anticlastogenic compounds with different chemical structure.

Ejchart A. **Adaptation of the cytokinesis-block technique for genotoxicity assay on L929 cell line.** Acta Pol Pharm 1997;54(6):433-5.
The in vitro micronucleus assay was used as a method for evaluation of chromosomal damage in L929 cells. Known genotoxins were used to induce micronuclei: bleomycin the direct acting agent, and cyclophosphamide the substance which requires metabolic activation. The cytokinesis-block technique and the use of exogenous metabolizing system is precisely described.

Elhajouji A, Cunha M, Kirsch-Volders M. **Spindle poisons can induce polyploidy by mitotic slippage and micronucleate mononucleates in the cytokinesis-block assay.** Mutagenesis 1998;13(2):193-8.
The human in vitro cytokinesis-blocked micronucleus (MN) assay has been extensively used for detection of clastogenic and aneugenic agents. In this test binucleate cells are generally considered to be the main target cell population for assessing genotoxic effect and almost no attention is paid to the biological information contained in mono-nucleate cells. In this study we analysed the frequencies of micronucleate mononucleates in a control population and after in vitro exposure to clastogens or aneugens. A clear increase in MN in mononucleates was found only after exposure to aneugenic compounds. By means of fluorescence in situ hybridization using a chromosome 1-specific probe we further analysed the proportion of mononucleate cells with and without MN which were tetrasomic (tetraploid) and would have been induced during aneugen treatment by mitotic slippage. The data indicate that treatment with nocodazole induces tetrasomy for chromosome 1 (tetraploidy) and an increase in MN frequency in mononucleate diploid and tetraploid lymphocytes. The results thus confirm that some mononucleates pass mitosis without chromatid segregation to daughter nuclei. These data suggest that MN in mononucleates may be useful to distinguish clastogens from aneugens and increase the sensitivity of the test.

Elliott BM, Mackay JM, Clay P, Ashby J. **An assessment of the genetic toxicology of antimony trioxide.** Mutat Res 1998;415(1-2):109-17.
Antimony trioxide (Sb2O3, CAS 1309-64-4) has been examined in a range of in vitro and in vivo genotoxicity assays. Negative results were obtained with the Salmonella/microsome assay and the L5178Y mutation assay, but a positive response was observed in the in vitro cytogenetic assay using isolated human peripheral lymphocytes. However, in vivo, antimony trioxide was non-clastogenic in the mouse bone marrow micronucleus assay, following oral gavage administration for 1, 7, 14 or 21 days at dose levels of up to 5000 mg/kg (single dose) or 1000 mg/kg (repeat dose). A negative result was also obtained in the in vivo rat liver DNA repair (unscheduled DNA synthesis) assay following a single oral gavage administration of doses up to 5000 mg/kg. These data show no genotoxicity for antimony trioxide in vivo and do not confirm a previous report of clastogenicity in the mouse on repeated dosing. It is concluded that antimony trioxide is not genotoxic in vivo and does not present a genotoxic hazard to humans.

Fahrig R, Rupp M, Steinkamp-Zucht A, Bader A. **Use of primary rat and human hepatocyte sandwich cultures for activation of indirect carcinogens: monitoring of DNA strand breaks and gene mutations in co-cultured cells.** Toxicol In Vitro 1998;12(4):431-44.
BIOSIS COPYRIGHT: BIOL ABS. Loss of cytochrome P-450 content is a common feature in conventional culture systems of primary hepatocytes. In contrast to the standard in vitro situation, in vivo each hepatocyte is exposed to

n extracellular matrix (space of Disse) at two opposing basolateral surfaces. This in vivo symmetry has been reconstructed in vitro by culturing rat or human hepatocytes within two layers of Collagen, thus forming a sandwich configuration. Activation of dimethylbenzanthracene (DMBA) or benzo(a)pyrene (BaP) was studied in rat and human hepatocytes. Genotoxic effects were studied in a three-dimensional co-culture model between sandwich hepatocytes and mammalian cells using the comet assay for detection of DNA strand breaks, and the HPRT test for detection of gene mutations. Sandwich hepatocytes generated active metabolites. The maintenance of metabolic properties in hepatocytes was dependent on extracellular matrix geometry. The number of DMBA- or BaP-induced genotoxic effects tended to be higher than in standard S-9 mix assays. While the ability to activate indirect carcinogens disappears within hours in primary hepatocytes, hepatocyte sandwich cultures enhance their ability to activate indirect carcinogens within 1 wk and retain this activity for up to 2 wk. This is the main advantage of the sandwich method over the more simple and conventional assays. While freshly isolated hepatocytes, regardless of whether in sandwich culture or in conventional assays, are injured by the isolation procedure and possess a corresponding reduced activation ability, hepatocytes in sandwich cultures recover over the course of a few days, and acquire a much higher ability to activate indirect carcinogens. Consequently, the indirect carcinogens BaP and DMBA, which were ineffective (BaP) or exhibited only weak effects (DMBA) at a concentration of 160 nmol/ml in 1-2-day-old hepatocytes, were clearly effective (BaP) or showed about a threefold increase in genotoxicity (DMBA) in 8-day-old hepatocytes in sandwich co-culture. In contrast to the experiments with S-9 mix, which is toxic to mammalian cells and does not allow treatment times of more than 2-3 hr, cells in co-culture with human or rat hepatocytes can be treated for at least 24 hr. The use of sandwich cultures has not yet been described for genotoxicity studies. The results of he present study may perhaps facilitate the acceptance of this method as a co-culture model for the field of genetic toxicology. Use of hepatocytes alone for genotoxicity studies cannot be recommended for difficulties in isolating intact cells from the sandwich cultures. The use of human hepatocytes in sandwich co-culture should enable a more relevant evaluation of potential human genotoxicity with specific chemicals and should put the extrapolation of genetic toxicology data from animal species to humans on a more scientific basis. Beyond that, experiments with animals in vivo could be avoided.

Fenech M. **Important variables that influence base-line micronucleus frequency in cytokinesis-blocked lymphocytes-a biomarker for DNA damage in human populations.** Mutat Res 1998;404(1-2):155-65.
The cytokinesis-block micronucleus (CBMN) assay has been adopted by numerous laboratories as a means for rapidly assessing base-line chromosome damage (breakage and loss) in human populations. However, the appropriate implementation of this assay requires a thorough understanding of both experimental variables and biological factors that can have impact on micronucleus (MN) frequency. The paper describes, with the help of experimental data the from the author's laboratory as well as other data, the impact of these variables. With regards to experimental variables, the scoring of micronuclei on slides by different technicians has been identified as an important factor; however, the use of different culture media, namely RPMI 1640 and McCoy's medium, did not have a significant effect on base-line frequencies. The paper also describes results showing that the MN index in cytokinesis-blocked cells, measured once every three months over a 12-month period for 53 healthy subjects, remains constant and the data measured on these occasions were significantly and positively correlated (R=0.477 to 0.684, P<0. 0001) with each other thus indicating the reliability and intra-individual variability of the assay over time. Inter-individual variation for males and female subjects has been estimated for each decade of age between 20 and 80 years; the difference between the 25th and 75th percentile of MN frequency varied between 1.4 fold and 2.3 fold and the minimum and maximum values for MN frequency varied by a factor of 4.7 and 12.5 depending on the age group. Age and gender are the most important demographic variables impacting on the MN index with MN frequencies in females being greater than those in males by a factor of 1.2 to 1.6 depending on the age group. For both sexes, MN frequency was significantly and positively correlated with age (R=0.62 in males and R=0.65 in females) and the slope of the regression line in males was 0.314 (P<0.0001) and in females it was 0.517 (P<0.0001). The main dietary factors influencing the MN index in subjects who are not folate deficient are plasma B12 (R=-0.315, P=0.0127) and plasma homocysteine (R=0.415, P=0.0086). In addition, it was proposed that the MN index is likely to be influenced by the propensity of an individual's cells to undergo apoptosis when damaged so that one might expect the MN frequency to be negatively correlated with apoptotic rate although this has yet to be tested. The above indicates the importance of maintaining an international network of scientists working with the CBMN assay to ensure appropriate quality control and for the development of standard experimental and

documentation protocols. The human micronucleus (HUMN) project launched in 1997 is briefly described and proposed as the vehicle for these activities. Copyright 1998 Elsevier Science B.V.

Galloway SM, Miller JE, Armstrong MJ, Bean CL, Skopek TR, Nichols WW. **DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens.** Mutat Res 1998;400(1-2):169-86.
Positive results in the in vitro assay for chromosome aberrations sometimes occur with test chemicals that apparently do not react with DNA, being negative in tests for mutation in bacteria, for DNA strand breaks, and for covalent binding to DNA. These chromosome aberrations typically occur over a narrow concentration range at toxic doses, and with mitotic inhibition. Indirect mechanisms, including oxidative damage, cytotoxicity and inhibition of DNA synthesis induced by chemical exposure, may be involved. Understanding when such mechanisms are operating is important in evaluating potential mutagenic hazards, since the effects may occur only above a certain threshold dose. Here, we used two-parameter flow cytometry to assess DNA synthesis inhibition (uptake of bromodeoxyuridine [BrdUrd]) associated with the induction of aberrations in CHO cells by DNA-reactive and non-reactive chemicals, and to follow cell cycle progression. Aphidicolin (APC), a DNA polymerase inhibitor, induces aberrations without reacting with DNA; 50 microM APC suppressed BrdUrd uptake during a 3-h treatment to <10% of control levels. Several new drug candidates induced aberrations concomitant with marked reductions in cell counts at 20 h (to 50-60% of controls) and suppression of BrdUrd uptake (<15% of control). Several non-mutagenic chemicals and a metabolic poison, which induce DNA double strand breaks and chromosome aberrations at toxic dose levels, also suppressed DNA synthesis. In contrast, the alkylating agents 4-nitroquinoline-1-oxide, mitomycin C, methylnitrosourea, ethylnitrosourea, methylmethane sulfonate and ethylmethane sulfonate, and a topoisomerase II inhibitor, etoposide, produced many aberrations at concentrations that were less toxic (cell counts >/=73% of controls) and gave little inhibition of DNA synthesis during treatment (BrdUrd uptake >/=85% of controls), although cell cycle delay was seen following the 3-h treatment. Thus, inhibition of DNA synthesis at the time of treatment is supporting evidence for an indirect mechanism of aberrations, when there is no direct DNA reactivity. Copyright 1998 Elsevier Science B.V. All rights reserved.

Garcia-Sanchez F, Pizzorno G, Fu SQ, Nanakorn T, Krause DS, Liang J, Adams E, Leffert JJ, Yin LH, Cooperberg MR, et al. **Cytosine deaminase adenoviral vector and 5-fluorocytosine selectively reduce breast cancer cells 1 million-fold when they contaminate hematopoietic cells: a potential purging method for autologous transplantation.** Blood 1998;92(2):672-82.
Ad.CMV-CD is a replication incompetent adenoviral vector carrying a cytomegalovirus (CMV)-driven transcription unit of the cytosine deaminase (CD) gene. The CD transcription unit in this vector catalyzes the deamination of the nontoxic pro-drug, 5-fluorocytosine (5-FC), thus converting it to the cytotoxic drug 5-fluorouracil (5-FU). This adenoviral vector prodrug activation system has been proposed for use in selectively sensitizing breast cancer cells, which may contaminate collections of autologous stem cells products from breast cancer patients, to the toxic effects of 5-FC, without damaging the reconstitutive capability of the normal hematopoietic cells. This system could conceivably kill even the nondividing breast cancer cells, because the levels of 5-FU generated by this system are 10 to 30 times that associated with systemic administration of 5-FU. The incorporation of 5-FU into mRNA at these high levels is sufficient to disrupt mRNA processing and protein synthesis so that even nondividing cells die of protein starvation. To test if the CD adenoviral vector sensitizes breast cancer cells to 5-FC, we exposed primary explants of normal human mammary epithelial cells (HMECs) and the established breast cancer cell (BCC) lines MCF-7 and MDA-MB-453 to the Ad.CMV-CD for 90 minutes. This produced a 100-fold sensitization of these epithelial cells to the effects of 48 hours of exposure to 5-FC. We next tested the selectivity of this system for BCC. When peripheral blood mononuclear cells (PBMCs), collected from cancer patients during the recovery phase from conventional dose chemotherapy-induced myelosuppression, were exposed to the Ad.CMV-CD for 90 minutes in serum-free conditions, little or no detectable conversion of 5-FC into 5-FU was seen even after 48 hours of exposure to high doses of 5-FC. In contrast, 70% of 5-FC was converted into the cytotoxic agent 5-FU when MCF-7 breast cancer cells (BCCs) were exposed to the same Ad.CMV-CD vector followed by 5-FC for 48 hours. All of the BCC lines tested were shown to be sensitive to infection by adenoviral vectors when exposed to a recombinant adenoviral vector containing the reporter gene betagalactosidase (Ad.CMV-betagal). In contrast, less than 1% of the CD34-selected cells and their more immature subsets, such as the CD34+CD38- or CD34(+)CD33-

subpopulations, were positive for infection by the Ad.CMV-betagal vector, as judged by fluorescence-activated cell sorting (FACS) analysis, when exposed to the adenoviral vector under conditions that did not commit the early hematopoietic precursor cells to maturation. When artificial mixtures of hematopoietic cells and BCCs were exposed for 90 minutes to the Ad.CMV-CD vector and to 5-FC for 10 days or more, a greater than 1 million fold reduction in the number of BCCs, as measured by colony-limiting dilution assays, was observed. To test if the conditions were damaging for the hematopoietic reconstituting cells, marrow cells collected from 5-FU-treated male donor mice were incubated with the cytosine deaminase adenoviral vector and then exposed to 5-FC either for 4 days in vitro before transplantation or for 14 days immediately after transplantation in vivo. There was no significant decrease in the reconstituting capability of the male marrow cells, as measured by their persistence in female irradiated recipients for up to 6 months after transplantation. These observations suggest that adenovirus-mediated gene transfer of the Escherichia coli cytosine deaminase gene followed by exposure to the nontoxic pro-drug 5-FC may be a potential strategy to selectively reduce the level of contaminating BCCs in collections of hematopoietic cells used for autografts in breast cancer patients.

Gentile JM, Gentile GJ, Nannenga B, Johnson M, Blankespoor H, Montero R. **Enhanced liver cell mutations in trematode-infected Big Blue transgenic mice.** Mutat Res 1998;400(1-2):355-60.
Parasite infections in humans have long been associated with specific types of cancers. Schistosoma hematobium is a known inducer of urinary bladder cancer, Helicobacter pylori is a gastric carcinogen, and hepatitis B virus and Opisthorchis viverrini are causative agents of liver cell cancers. Another liver fluke, Fasciola hepatica, has also been identified as a neoplastic risk agent, primarily in animals. We used F. hepatica as a model agent to determine if the presence of an aggressive liver fluke could induce mutagenic events in mammalian tissue. Using the Big Blue(R) transgenic mouse assay, we found a two-fold increase in lacI mutations in cells harvested from mice harboring F. hepatica worms when compared to uninfected control animals. These data indicate that biological infections can cause increased genetic damage in surrounding host tissue.

Gibson DP, Ma X, Switzer AG, Murphy VA, Aardema MJ. **Comparative genotoxicity of quinolone and quinolonyl-lactam antibacterials in the in vitro micronucleus assay in Chinese hamster ovary cells.** Environ Mol Mutagen 1998;31(4):345-51.
The in vitro micronucleus assay is gaining increased attention as a potential alternative to the standard in vitro metaphase analysis assay. In particular, the in vitro micronucleus assay has been proposed as a useful method for chemicals that induce both structural and numerical chromosome alterations, such as DNA gyrase/topoisomerase inhibitors. In this study, we compared the micronucleus-inducing activity of quinolonyl-lactam antibacterials that inhibit DNA-gyrase and bind to penicillin-binding proteins relative to the activity of structurally related quinolone antibacterials that also inhibit DNA-gyrase. All of the quinolones that were structurally related to the quinolonyl-lactams were cytotoxic and induced large increases in the frequency of micronucleated binucleated cells (MNBC) at concentrations between 0.02 and 0.16 mM. These changes were larger than those seen with the commercial quinolones, ciprofloxacin (cytotoxic at > or = 0.57 mM and MNBC at > or = 0.3 mM) and nalidixic acid (cytotoxic at 1.8 mM and no MNBC up to this dose). In contrast, the quinolonyl-lactams were not cytotoxic up to 1.0 mM concentrations and induced either no MNBC or a low frequency of MNBC at higher concentrations compared to the quinolones. Quinolonyl-lactams appear to be less cytotoxic and genotoxic than structurally related quinolones. These results add to the growing database on the in vitro micronucleus assay in general, and more specifically to the relatively small database for the in vitro micronucleus assay in Chinese hamster ovary cells.

Gichner T, Plewa MJ. **Induction of somatic DNA damage as measured by single cell gel electrophoresis and point mutation in leaves of tobacco plants.** Mutat Res 1998;401(1-2):143-52.
The induction and measurement of DNA damage in nuclei of plant tissues is a new area of study with the alkaline single cell gel electrophoresis/comet assay. Methods to isolate plant cell nuclei cause high levels of DNA damage which are detected by the comet assay. We developed a method to isolate nuclei from leaf tissue of Nicotiana tabacum (a1+/a1; a2+/a2) in a modified Sorensen buffer that resulted in constant, low tail moment values for the negative controls. After treating intact tobacco plants with 1-8 mM ethyl methanesulfonate (EMS) we obtained a

direct concentration-response with an average median tail moment of 65.9+/-4.4 micro(m) for plants exposed to the highest EMS concentration as compared to the median control tail moment value of 4.1+/-0.8. We found that the highest resolution was obtained with electrophoretic conditions of 0.74 V/cm at 300 mA for 20 min. Multiple leaves could be analyzed per plant within each treatment group and the tail moments were not significantly different. Tobacco seedlings were treated with EMS in the same manner as used for the comet assay and mutations were induced in the leaf primordia. The mean mutant frequency for the control was 1.46+/-0.20 mutant sectors/leaf. The mutant frequency increased in a concentration dependent manner; the mutant frequency induced by 8 mM EMS was 37.89+/-2.37 mutant sectors/leaf. The comet tail moment values and the leaf mutant frequency were highly correlated (r=0.98). The genetic response factor was calculated by the ratio of the difference in the response within the linear portion of each concentration-response curve divided by the slope of the curve. The genetic response factor for the tail moment was 7.82 while the value for mutation induction was 7.76. In this paper we describe a sensitive method with high resolution to apply the alkaline comet assay to plant leaves. The comet assay response was compared to that of induced point mutation. With this sensitive method for nuclei isolation from plant leaves, the alkaline SCGE assay could be incorporated into in situ plant environmental monitoring.

Goeger DE, Anderson KE, Hsie AW. **Coumarin chemoprotection against aflatoxin B1-induced gene mutation in a mammalian cell system: a species difference in mutagen activation and protection with chick embryo and rat liver S9.** Environ Mol Mutagen 1998;32(1):64-74.
Coumarin (1,2-benzopyrone), a natural food constituent, prevents polycyclic aromatic hydrocarbon-induced neoplasms in rats and mice, but has not been studied with other chemical carcinogens. We examined coumarin chemoprotection against aflatoxin B1 using the 6-thioguanine resistance mutation assay in two different Chinese hamster ovary cell lines (K1BH4 and AS52) with liver S9 from rats and 19-day-old chick embryos for aflatoxin B1 bioactivation. Laboratory rodents metabolize coumarin through 3-hydroxylation, whereas 7-hydroxylation predominates in chick embryos and humans. Chick embryo liver S9 was approximately 25-fold more effective in activating aflatoxin B1 to the mutagenic and cytotoxic metabolite(s) than rat liver S9. Coumarin added at 50 and 500 microM with chick embryo liver S9 reduced the mutant frequency of 1 microM aflatoxin B1 by 40 and 85%, respectively. Coumarin up to 500 microM had no effect on aflatoxin B1 mutagenicity with rat liver S9. When liver S9 from chick embryos pretreated with coumarin was used for aflatoxin B1 bioactivation, mutant frequency and cytotoxicity were decreased compared to liver S9 from vehicle-treated controls. Liver S9 from coumarin-treated rats did not significantly affect mutant frequency or cytotoxicity. HPLC analysis of chick embryo liver S9 incubated with 1 microM aflatoxin B1 showed a dose-dependent decrease by coumarin of aflatoxin B1 activation to the 8,9-epoxide ranging from 70% of controls at 5 microM coumarin to 4% of controls at 500 microM coumarin. In contrast, coumarin produced a dose-dependent increase in 20 microM aflatoxin B1 activation by rat liver S9, reaching twice the control levels at 500 microM coumarin. These findings, using a mammalian cell system as a mutagenic endpoint, demonstrate marked species differences in chemoprotection by coumarin.

Gomes-Carneiro MR, Felzenszwalb I, Paumgartten FJ. **Mutagenicity testing (+/-)-camphor, 1,8-cineole, citral, citronellal, (-)-menthol and terpineol with the Salmonella/microsome assay.** Mutat Res 1998;416(1-2):129-36.
The essential oils and their monoterpenoid constituents have been widely used as fragrances in cosmetics, as flavouring food additives, as scenting agents in a variety of household products, as active ingredients in some old drugs, and as intermediates in the synthesis of perfume chemicals. The present study was undertaken to investigate the mutagenic potential of six monoterpenoid compounds: two aldehydes (citral and citronellal), a ketone ((+/-)-camphor), an oxide (1,8-cineole, also known as eucalyptol), and two alcohols (terpineol and (-)-menthol). It is part of a more comprehensive toxicological screening of monoterpenes under way at our laboratory. Mutagenicity was evaluated by the Salmonella/microsome assay (TA97a, TA98, TA100 and TA102 tester strains), without and with addition of an extrinsic metabolic activation system (lyophilized rat liver S9 fraction induced by Aroclor 1254). In all cases, the upper limit of the dose interval tested was either the highest non-toxic dose or the lowest dose of the monoterpene toxic to TA100 strain in the preliminary toxicity test. No mutagenic effect was found with (+/-) camphor, citral, citronellal, 1,8-cineole, and (-) menthol. Terpineol caused a slight but dose-related increase in the number of his+ revertants with TA102 tester strain both without and with addition of S9 mixture. The results from

this study therefore suggest that, with the exception of terpineol, the monoterpenoid compounds tested are not mutagenic in the Ames test.

Graf U, Abraham SK, Guzman-Rincon J, Wurgler FE. **Antigenotoxicity studies in Drosophila melanogaster.** Mutat Res 1998;402(1-2):203-9.
The fruit fly Drosophila melangaster with its well developed array of genotoxicity test systems has been used in a number of studies on antigenotoxicity of various compounds and mixtures. In recent years, the newly developed Somatic Mutation and Recombination Tests (SMART) have mainly been employed. These one-generation tests make use of the wing or eye imaginal disc cells in larvae and have proven to be very efficient and sensitive. They are based on the principle that the loss of heterozygosity of suitable recessive markers can lead to the formation of mutant clones of cells that are then expressed as spots on the wings or eyes of the adult flies. We have employed the wing spot test with the two markers multiple wing hairs (mwh,3-0.3) and flare (flr,3-38.8). Three-day-old larvae, trans-heterozygous for these markers, are treated chronically or acutely by oral administration with the test compound(s) or complex mixtures. For antigenotoxicity studies, chronic co-treatments can be used, as well as separate pre-treatments with an antigenotoxic agent followed by a chronic treatment with a genotoxin. After eclosion, the wings of the adult flies are scored for the presence of single and twin spots. These spots can be due to different genotoxic events: either mitotic recombination or mutation (deletion, point mutation, specific types of translocation, etc.). The analysis of two different genotypes (one with structurally normal chromosomes, one with a multiply inverted balancer chromosome) allows for a quantitative determination of the recombinagenic activity of genotoxins. Results of two separate studies presented: (1) instant coffee has antirecombinagenic but not antimutagenic activity in the wing spot test; and (2) ascorbic acid and catechin are able to protect against in vivo nitrosation products of methyl urea in combination with sodium nitrite.

Grant WF, Owens ET. **Chromosome aberration assays in Crepis for the study of environmental mutagens.** Mutat Res 1998;410(3):291-307.
Crepis capillaris (2n=6) is an excellent plant for the assay of chromosome aberrations after chemical treatment. C. tectorum (2n=8) has been used also in mutagenic studies, but to a much lesser extent. A protocol has been given for using root tips to study the cytological endpoints, such as chromosome breaks and exchanges, which follow the testing of chemicals in somatic cells. Meiotic endpoints have not been used in C. capillaris for testing potential chemical mutagens but should be considered, especially a meiotic micronucleus assay. From a literature survey, 81 chemicals are tabulated that have been assayed in 162 Crepis assays for their clastogenic effects. Of the 162 assays that have been carried out, 40 are reported at giving a positive reaction (i.e., causing chromosome aberrations), 97 positive and with a dose response, 7 borderline positive, and 17 negative. Eighty-five percent of the chemicals gave a definite positive response. Assays for one chemical gave contrary results, and were not included in the above tabulation. The Crepis bioassay has been shown to be an excellent plant bioassay for assessing chromosome damage induced by chemicals and environmental pollutants.

Hartmann A, Fender H, Speit G. **Comparative biomonitoring study of workers at a waste disposal site using cytogenetic tests and the comet (single-cell gel) assay.** Environ Mol Mutagen 1998;32(1):17-24.
Workers exposed to environmental pollutants at a waste disposal site were studied for genotoxic effects with cytogenetic tests and the comet (alkaline single-cell gel) assay. Analyses were performed on peripheral blood samples of 44 workers at a waste disposal site (DM) and 47 subjects of a control group (VE) matched for gender, age, and smoking habits. Chromosomal aberrations were evaluated in 1,000 lymphocytes per individual, sister chromatid exchanges in 50 cells, and DNA migration (tail moment) was determined in 100 leukocytes. Structural chromosome aberrations were more frequent in DM than in VE, but only the frequency of acentric fragments and the percentage of aberrant cells (excluding gaps) was significantly increased. No significant difference was found for the mean frequency of SCE. A statistically significant difference was also seen with the comet assay. The mean tail moment was higher in DM than in VE. However, no correlation was found between cytogenetic data and the effects in the comet assay. The results of our study indicate that DNA effects in the comet assay represent an

independent endpoint which might be useful for the biomonitoring of genotoxic effects in addition to established tests.

Hei TK, Liu SX, Waldren C. **Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species.** Proc Nat Acad Sci U S A 1998;95(14):8103-7.
BIOSIS COPYRIGHT: BIOL ABS. Arsenite, the trivalent form of arsenic present in the environment, is a known human carcinogen that lacked mutagenic activity in bacterial and standard mammalian cell mutation assays. We show herein that when evaluated in an assay (AL cell assay), in which both intragenic and multilocus mutations are detectable, that arsenite is in fact a strong dose-dependent mutagen and that it induces mostly large deletion mutations. Cotreatment of cells with the oxygen radical scavenger dimethyl sulfoxide significantly reduces the mutagenicity of arsenite. Thus, the carcinogenicity of arsenite can be explained at least in part by it being a mutagen that depends on reactive oxygen species for its activity.

Helleday T, Arnaudeau C, Jenssen D. **Effects of carcinogenic agents upon different mechanisms for intragenic recombination in mammalian cells.** Carcinogenesis 1998;19(6):973-8.
A growing body of carcinogens are known to affect genetic recombination in mammalian cells and to thereby interfere with the process of carcinogenesis. In order to screen for recombinogenic effects of chemical and physical agents a variety of in vitro assay systems utilizing mammalian cells have been developed. However, the effects of potential carcinogens differ in these different systems. In order to investigate this phenomenon further, we have employed two different assay procedures, involving spontaneous duplication mutants in mammalian cells, which respond to homologous or non-homologous recombination. Four carcinogens were investigated, i.e. Aroclor 1221, benzene, methylmethanesulphonate (MMS) and thiourea, as were gamma- and UV-irradiation. With the exception of thiourea all of these factors resulted in elevated frequencies of homologous recombination. On the other hand, only UV-irradiation affected the rate of non-homologous recombination. These results indicate that substrate length and/or the recombination mechanism may influence the recombinogenic response of mammalian fibroblasts to carcinogenic factors. Thus, procedures for recombinogenic effects of carcinogens should consider the different pathways of recombination occurring in mammalian cells.

Herrero ME, Arand M, Hengstler JG, Oesch F. **Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide but not from ethylene oxide-induced DNA strand breaks.** Environ Mol Mutagen 1997;30(4):429-39.
The impact of human-microsomal-epoxide-hydrolase (hmEH) on the genotoxic effects of styrene-7,8-oxide (96093) (STO) and ethylene-oxide (75218) (EO) was examined. V79 Chinese-hamster cells were cultured and transfected with the pMPSV/hmEH expression vector and the pPUR selection vector using the calcium-phosphate precipitation technique. Mock transfected cells were transfected with the pPUR plasmid only. The S9 fractions of the puromycin resistant clones were analyzed for hmEH expression via immunoblotting. The hmEH activity of the clones was determined using STO as the substrate. Indirect immunofluorescence staining visualized the distribution of hmEH in the transfected cells. Mock and hmEH transfected cells treated with varying concentrations of STO or EO were examined for DNA damage by alkaline elution analysis. The effect of STO exposure on cloning efficiency was also determined. According to immunoblot analysis, one in three cell clones expressed enzymatically active hmEH at high levels. The hydrolysis of STO was increased significantly in hmEH transfected cells, compared to mock transfected cells. Within clone 92, which had the strongest hmEH protein expression and enzymatic activity, over 80% of the cells contained high levels of hmEH. Within the individual cells of this clone, hmEH appeared to be associated with the endoplasmic reticulum. The STO doses causing DNA damage in V79 cells were significantly lower in hmEH transfected cells than in mock transfected cells. Survival rates of V79 cells treated with STO were significantly higher in hmEH transfected cells than in mock transfected cells. The protective effect of hmEH was blocked in the presence of valpromide, a competitive inhibitor of hmEH. EO induced DNA damage did not differ between hmEH transfected and mock transfected cells. The authors conclude that hmEH protects against the genotoxic effects of STO, but not EO.

Hilliard CA, Armstrong MJ, Bradt CI, Hill RB, Greenwood SK, Galloway SM. **Chromosome aberrations in vitro**

related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. Environ Mol Mutagen 1998;31 (4):316-26.
BIOSIS COPYRIGHT: BIOL ABS. Chromosome aberrations can occur by secondary mechanism(s) associated with cytotoxicity, induced by chemicals that do not attack DNA. Aberrations are formed From DNA double-strand breaks, and DSBs are known to be induced by nonmutagenic (Ames test negative) noncarcinogens at toxic levels (Storer et al. (1996): Mutat Res 368:59-101). Here, 8 of 12 of these chemicals caused aberrations in CHO cells at cytotoxic doses, and often only when cell counts (survival) at 20 hr approached :50% of controls. Five of eight noncarcinogens (2,4,-dichlorophenol, dithiocarb, menthol, phthalic anhydride, and ethionamide) and one of two equivocal carcinogens (bisphenol A) caused aberrations, usually over a narrow dose range with steeply increasing cytotoxicity. Phthalic anhydride and ethionamide were positive only at doses with precipitate. Phenformin was negative even at toxic doses and ephedrine and phenylephrine were negative and gave little toxicity. Aberrations were also induced by metabolic poisons, 2,4-dinitrophenol, (uncouples oxidative phosphorylation), and sodium iodoacetate, (NaI; blocks ATP production). Five of the chemicals that induced aberrations in CHO cells were tested in human TK6 cells and four were positive, the fifth being equivocal. Stable aberrations (translocations) were induced in human cells by NaI. Clearly, chemicals can give "false-positive" results in the chromosome aberration assay at cytotoxic levels, though cytotoxicity does not always produce aberrations, so that further information (e.g., DNA reactivity) is needed to determine whether a result is a "false-positive." Primary DNA-damaging chemicals such as alkylators are also cytotoxic, but give strong increases in aberrations without marked initial toxicity by the measures used here, although the aberrations they induce do reduce long-term survival in colony-forming assays.

Hughes CM, Lewis SE, McKelvey-Martin VJ, Thompson W. **The effects of antioxidant supplementation during Percoll preparation on human sperm DNA integrity.** Hum Reprod 1998;13(5):1240-7.
The integrity of sperm DNA is crucial for the maintenance of genetic health. A major source of damage is reactive oxygen species (ROS) generation; therefore, antioxidants may afford protection to sperm DNA. The objectives of the study were, first, to measure the effects of antioxidant supplementation in vitro on endogenous DNA damage in spermatozoa using the single cell gel electrophoresis (comet) assay and, second, to assess the effect of antioxidant supplementation given prior to X-ray irradiation on induced DNA damage. Spermatozoa from 150 patients were prepared by Percoll centrifugation in the presence of ascorbic acid (300, 600 microM), alpha tocopherol (30, 60 microM), urate (200, 400 microM), or acetyl cysteine (5, 10 microM). DNA damage was induced by 30 Gy X-irradiation. DNA strand breakage was measured using the comet assay. Sperm DNA was protected from DNA damage by ascorbic acid (600 microM), alpha tocopherol (30 and 60 microM) and urate (400 microM). These antioxidants provided protection from subsequent DNA damage by X-ray irradiation. In contrast, acetyl cysteine or ascorbate and alpha tocopherol together induced further DNA damage. Supplementation in vitro with the antioxidants ascorbate, urate and alpha tocopherol separately has beneficial effects for sperm DNA integrity.

Ishidate M Jr, Miura KF, Sofuni T. **Chromosome aberration assays in genetic toxicology testing in vitro.** Mutat Res 1998;404(1-2):167-72.
The chromosome aberration test using cultured mammalian cells is one of the sensitive methods to predict environmental mutagens and/or carcinogens, and is a complementary test to the Salmonella/microsome assay (Ames test). From our recent survey of 951 chemicals which have been tested for their clastogenicity in cultured mammalian cells such as Chinese hamster fibroblasts or human lymphocytes, it was noted that 47% of them are consistently positive either with or without metabolic activation. When the test was performed using the cell line CHL/IU, 39.2% (292/745) were found to be positive. However, 8% (36/447) of such clastogens were positive only at an extremely high concentration of more than 10 mM. About 11% (48/447) of clastogens such as diethylstilbestrol (DES) and methyl AalphaC (Glob-P-1) induced mainly polyploid cells. Most chemicals induced chromatid-type aberrations, some induce only break-type aberrations at relatively high dose levels, but others induce more exchange-type aberrations at relatively low dose levels. Clastogenic activities were compared among different clastogens, using the D20 value, which is the minimum dose (mg/ml) at which aberrations were found in 20% of metaphases. In addition, the translocation (TR) value was calculated from the incidence of cells with exchange-type aberrations. It was suggested that possible carcinogens are included in the group of compounds with relatively low D20 values, but with high TR values. Karyological analysis was performed, using a FISH painting probe prepared

from No. 1 chromosome of CHO cells, on the clonal subline isolated after treatment with benzo(a)pyrene. However, no specific changes common to the agent were detected. Laser scanning cytometry (LSC) was also applied to screen for abnormal karyotypes. A translocation between particular chromosomes was reflected by the deletion of a DNA peak. Copyright 1998 Elsevier Science B.V.

Jenkins GJ, Chaleshtori MH, Song H, Parry JM. **Mutation analysis using the restriction site mutation (RSM) assay.** Mutat Res 1998;405(2):209-20.
The restriction site mutation (RSM) assay (see Steingrimsdottir et al. [H. Steingrimsdottir, D. Beare, J. Cole, J.F.M. Leal, T. Kostic, J. Lopez-Barea, G. Dorado, A.R. Lehmann, Development of new molecular procedures for the detection of genetic alteration in man, Mutat. Res. 353 (1996) pp. 109-121] for a review) has been developed as a genotypic mutation detection system capable of identifying mutations occurring in restriction enzyme sites of genomic DNA. Here we will report the steps taken to overcome some of the initial problems of the assay, namely the lack of quantitative data and limited sensitivity, the aim being to achieve a methodology suitable for the study of low dose chemical exposures. Quantitative data was achieved in the RSM assay by the inclusion of an internal standard molecule in the PCR amplification stage, thus allowing the calculation of both spontaneous and induced mutation frequencies. The sensitivity of the assay was increased through the discovery that intron sequences of genomic DNA accumulated more mutations in vivo compared to the exons, presumably due to differential selective pressure within genes [G.J.S. Jenkins, I.deG. Mitchell, J.M. Parry, Enhanced restriction site mutation (RSM) analysis of 1, 2-dimethylhydrazine-induced mutations, using endogenous p53 intron sequences, Mutagenesis 12 (1997) pp. 117-123]. This increased sensitivity was examined by applying the RSM assay to analyse the persistence of N-ethyl-N-nitrosourea (ENU)-induced mutations in mice testes. Germ line mutations were sought in testes DNA 3, 10 and 100 days after ENU treatment. Mutations were detected in exons and especially intron regions, the intron mutations were more persistent, still being detected 100 days post-chemical treatment. Assignment of these mutations as ENU induced was complicated in some cases where the spontaneous mutation level was high. This theme of mutation persistence was further investigated by studying the presence of 4-nitroquinoline-1-oxide (4-NQO)-induced DNA mutations in vitro. This study also analysed the relationship between DNA adduct formation and DNA mutation induction by the concurrent RSM analysis and 32P post-labelling analysis of 4-NQO treated human fibroblasts. The results demonstrated that early DNA mutations detected 4 days post-treatment by the RSM assay were probably ex vivo mutations induced by Taq polymerase misincorporation of 4-NQO adducted DNA, due to the maximum levels of 4-NQO adducts being present at this time point. A later mutational peak, after the adduct level had declined, was assumed to be due to DNA sequence changes produced in the fibroblasts by the in vivo processing of DNA adducts. Copyright 1998 Elsevier Science B.V.

Johnson DF, Prezant TR, Lubavin B, Chaltraw WE, Fischel-Ghodsian N. **Isolation of overexpressed yeast genes which prevent aminoglycoside toxicity.** Hear Res 1998;120(1-2):62-8.
Aminoglycoside antibiotics at non-toxic levels can cause sensorineural hearing loss in genetically predisposed individuals. The major aminoglycoside hypersensitivity mutation that has been described in humans is at position 1555 in the mitochondrial 12S ribosomal RNA gene. In order to identify additional candidate genes for genetic susceptibility mutations in humans and possibly develop therapeutic interventions, we are using yeast as a model organism to identify genes whose products interact with aminoglycosides or bypass the effects of aminoglycoside poisoning. We have selected yeast genomic DNAs that, when cloned into a high copy number plasmid, confer neomycin resistance. We have previously described the first gene identified through this approach [Prezant, Chaltraw and Fischel-Ghodsian, Microbiology 142 (1996) 3407 3414] and have now completed this search by the exhaustive screening of 35 yeast genome equivalents. This has resulted in the identification of seven additional chromosomal regions. All seven chromosomal regions have been characterized and the most likely gene responsible for aminoglycoside resistance has been identified for each of them. While the mechanism of aminoglycoside resistance can be inferred for some of the gene products, it remains to be determined for others.

Johnson NF, Jaramillo RJ. **p53, Cip 1, and Gadd153 expression following treatment of A549 cells with natural and man-made vitreous fibers.** Environ Health Perspect 1997;105(Suppl 5):1143-5.
Studies were conducted to determine whether crocidolite (12001284) could induce DNA damage inducible genes. A

glass microfiber (JM-100) was used for comparison. Temporal and dose related expressions of p53, Cip-1, and Cadd153 proteins were determined in cultured A549 cells treated with either crocidolite or JM-100 and then cultured in fresh media. Cells were incubated with crocidolite or JM-100 at 5, 10, 15, 25 or 50 micrograms/milliliter for 20 hours, and then cultured in fresh medium for 8 days. Crocidolite induced the expression of all three proteins with a maximum expression after about 18 hours. At the same time point JM-100 did not markedly induce the three proteins. A dose dependent increase in the number of cells in the G2 phase of the cell cycle was also induced by crocidolite. Crocidolite has a high iron content compared to JM-100 which may be a factor in the expression of the proteins, but not a factor in the cytotoxic potential of the fibers, as JM-100 is more cytotoxic than crocidolite. The durability of crocidolite in the lung is much higher than that of JM-100, but biopersistence was not seen as a likely factor in the differences in protein response as the duration of the culture was only a small fraction of the half life of each of these materials. The authors suggest that crocidolite asbestos behaves similarly to ionizing radiation and genotoxic chemicals in its induction of proteins associated with DNA damage and cell cycle arrest. The difference in response between crocidolite and JM-100 may assist in understanding the mechanism of action of toxic and nontoxic fibers.

Kirkland DJ, Clements J. **Recommendations for spacing of test chemical concentrations in the mouse lymphoma tk mutation assay (MLA) [see comments].** Mutat Res 1998;415(1-2):159-63.
Recent test guidelines for the mouse lymphoma tk mutation assay (MLA) have highlighted the need to achieve 80-90% reduction in cell survival for a valid, robust assay with toxic chemicals. For many pharmaceuticals, under new ICH recommendations, this may be the only in vitro mammalian cell test that is performed. It was important to discover, therefore, how critical it is to achieve 80-90% toxicity, and how best to select the number and spacing of test concentrations to fulfil this requirement. We analysed data from 121 positive chemicals, provided by nine industrial and commercial laboratories, and found that for 17 chemicals (14%), the response profiles were so steep that using a conventional 2-fold dilution series of test concentrations would have failed to identify the active range (> 90% toxicity at one concentration, and no significant mutation at 50% of this dose), and positive responses would have been missed. Analysis of genotoxicity results in other test systems with these 17 chemicals revealed no differences in overall response profiles from the 104 chemicals that exhibited less steep MLA responses. The MLA results were therefore deemed to be equally biologically relevant. From this analysis, it is recommended that concentration spacing in the MLA needs to be closer than that obtained with a 2-fold dilution series, and a dilution factor where each concentration is 0.75 or 0.8 of the one above is recommended to identify the active range of positive mutagens.

Kitamura T. **New experimental approaches in retrovirus-mediated expression screening.** Int J Hematol 1998;67(4):351-9.
We have established an expression screening system that utilizes retrovirus-mediated gene transfer. The system is based on a high-efficiency packaging cell line BOSC23 and retrovirus vectors pBabeX and pMX which are suitable for cDNA library construction. The combination of BOSC23 packaging cells and the pBabeX and pMX retrovirus vectors produces high-titer recombinant retroviruses, which are able to cover large complexities of cDNA libraries. These retroviruses gave 100% infection efficiency in NIH3T3 cells and 10-100% infection efficiency in various hemopoietic cell lines. In contrast to the conventional mammalian expression cloning system where it is required to transduce cDNA transiently into particular cell types such as COS cells, the retrovirus-mediated expression cloning method allows stable transduction of cDNAs into a wide variety of cell types. This method, therefore, makes it possible to select cells expressing the desired cDNA by various functional assays. In addition, when combined with PCR-driven random mutagenesis, this system is also useful in searching for mutations of various molecules which will result in functional alterations. Other potential applications will also be discussed.

Klotz EL, Hackett J Jr, Storb U. **Somatic hypermutation of an artificial test substrate within an Ig kappa transgene.** J Immunol 1998;161(2):782-90.
We have characterized a novel substrate for somatic hypermutation, confirming that non-Ig sequences can be targeted for mutation and demonstrating that this substrate allows for the rapid assay for mutations. An artificial sequence containing alternating EcoRV and PvuII sites (EPS) was inserted into the Vkappa167 transgene, which is

known to be a target for mutation. To assay for somatic hypermutation, the EPS is amplified using flanking transgene primers, and the PCR product is subsequently digested with either EcoRV or PvuII. A mutation is seen as the appearance of a larger fragment, indicating a base change in a restriction enzyme site. The original transgene, Vkappa167/EPS, showed evidence of a low level of mutation in both splenic hybridomas and Peyer's patch-derived or immunized splenic B220+ cells with high peanut agglutinin levels. Two derivative lines of Vkappa167/EPS were made, Vkappa167/POX and Vkappa167/PEPS. While none of the Vkappa167/POX transgenic lines demonstrated mutation, the Vkappa167/PEPS transgene was highly mutated in B220+ splenic B cells with high peanut agglutinin levels at a frequency similar to that of endogenous Ig genes. An analysis of splenic RNA from the unimmunized transgenic mice indicated that the levels of stable message in splenic B cells could not be correlated with the mutation seen in GC B cells. The mutable Vkappa167/PEPS transgenic line is a unique tool to study somatic hypermutation in vivo.

Knasmuller S, Parzefall W, Sanyal R, Ecker S, Schwab C, Uhl M, Mersch-Sundermann V, Williamson G, Hietsch G, Langer T, et al. **Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens.** Mutat Res 1998;402(1-2):185-202.
The human hepatoma line (Hep G2) has retained the activities of various phase I and phase II enzymes which play a crucial role in the activation/detoxification of genotoxic procarcinogens and reflect the metabolism of such compounds in vivo better than experimental models with metabolically incompetent cells and exogenous activation mixtures. In the last years, methodologies have been developed which enable the detection of genotoxic effects in Hep G2 cells. Appropriate endpoints are the induction of 6-TGr mutants, of micronuclei and of comets (single cell gel electrophoresis assay). It has been demonstrated that various classes of environmental carcinogens such as nitrosamines, aflatoxins, aromatic and heterocyclic amines and polycyclic aromatic hydrocarbons can be detected in genotoxicity assays with Hep G2 cells. Furthermore, it has been shown that these assays can distinguish between structurally related carcinogens and non-carcinogens, and positive results have been obtained with rodent carcinogens (such as safrole and hexamethylphosphoramide) which give false negative results in conventional in vitro assays with rat liver homogenates. Hep G2 cells have also been used in antimutagenicity studies and can identify mechanisms not detected in conventional in vitro systems such as induction of detoxifying enzymes, inactivation of endogenously formed DNA-reactive metabolites and intracellular inhibition of activating enzymes.

Knutson A, Hellman P, Akerstrom G, Westin G. **Characterization of the human Megalin/LRP-2 promoter in vitro and in primary parathyroid cells.** DNA Cell Biol 1998;17(6):551-60.
The gp330/Megalin/LRP-2 protein belongs to the low-density lipoprotein receptor gene family and is believed to function as an endocytic receptor for the uptake of lipoproteins and many other ligands. Other functions of this protein may include a role in calcium sensing in the parathyroid glands and other tissues. In order to study the transcriptional regulation of the human LRP-2 gene, a clone containing the 5'-flanking region was isolated from a genomic DNA library, and a transient transfection protocol for primary bovine parathyroid cells was established. RNA mapping techniques located the transcriptional start site 136 bp upstream of the initiation codon. Transient expression in several cell types, including primary parathyroid cells, and in vitro transcription in HeLa cell nuclear extracts showed that sequences between -120 and -35 were important for activated transcription. This region contains consensus binding sites (GC boxes) for transcription factor Sp1. Mutation of the GC boxes abolished binding of Sp1 in vitro and resulted in reduced transcription in vitro and in transfected cells. Furthermore, Sp1 stimulated transcription when tethered to the LRP-2 core promoter through a heterologous DNA-binding domain. Through site-directed mutagenesis, we identified a novel atypical TATA element with the sequence TAGAAAA. Intriguingly, this sequence motif was shown previously not to mediate transcription in a systematic mutational analysis of the TATA motif. Possible roles of this novel TATA element in the regulation of transcription initiation are discussed. The isolation and characterization of the LRP-2 promoter and the 5'-flanking region and the establishment of a transient expression assay in primary parathyroid cells will facilitate studies on the regulatory mechanisms of the LRP-2 gene and of other genes expressed in the parathyroid glands.

Kong Q, Harris RS, Maizels N. **Recombination-based mechanisms for somatic hypermutation.** Immunol Rev

1998;162:67-76.
We review some experiments designed to test recombination-based mechanisms for somatic hypermutation in mice, particularly mechanisms involving templated mutation or gene conversion. As recombination and repair functions are highly conserved among prokaryotes and eukaryotes, pathways of mutation in microorganisms may prove relevant to the mechanism of somatic hypermutation. Escherichia coli initiates a recombination-based pathway of mutation in response to environmental stimuli, and this "adaptive" pathway of mutation has striking similarities with somatic hypermutation, as does a process of mutagenic repair that occurs at double-strand breaks in Saccharomyces cerevisiae. We present a model for recombination-based hypermutation of the immunoglobulin loci which could result in either templated or non-templated mutation.

Kramer PJ. **Genetic toxicology [see comments].** J Pharm Pharmacol 1998;50(4):395-405.
Systems for testing genetic toxicology are components of carcinogenic and genetic risk assessment. Present routine genotoxicity-testing is based on at least 20 years of development during which many different test systems have been introduced and used. Today, it is clear that no single test is capable of detecting all genotoxic agents. Therefore, the usual approach is to perform a standard battery of in-vitro and in-vivo tests for genotoxicity. Work-groups of the European Union (EU), the Organization for Economic Co-operation and Development (OECD), and, very recently, the work-group of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) have defined such standard battery tests. These and some currently used supplementary or confirmatory tests are briefly discussed here. Additional test systems for the assessment of genotoxic and carcinogenic hazard and risk are seriously needed. These tests must be more relevant to man than are current assays and less demanding in respect of cost, time and number of animals. Another aspect for reassessment derives from the actual situation in the pharmaceutical industry. Companies have to prepare for the world economy of the 21st century. Therefore, pharmaceutical research is speeding up tremendously by use of tools such as genomics, combinatorial chemistry, high throughput screening and proteomics. Toxicology and genotoxicology need to re-evaluate their changing environment and must find ways to respond to these needs. In conclusion, genetic toxicology needs to answer questions coming from two major directions: hazard and risk identification and high throughput testing.

Kranendonk M, Mesquita P, Laires A, Vermeulen NP, Rueff J. **Expression of human cytochrome P450 1A2 in Escherichia coli: a system for biotransformation and genotoxicity studies of chemical carcinogens.** Mutagenesis 1998;13(3):263-9.
BIOSIS COPYRIGHT: BIOL ABS. In this study we describe the development of strain BMX100, a new Escherichia coli K12 tester strain, derived from MX100, a strain which was constructed for detection of mutagens and for mechanistic studies of chemical carcinogens. We demonstrate here that strain BMX100 can be used for stable expression of human CYP1A2 or human CYP1A2 fused to rat liver NADPH cytochrome P450 reductase. Mutagenicity of precarcinogens known to be bioactivated by CYP1A2, namely 2-aminoanthracene (2-AA), aflatoxin B1 (AFB1) and 2-amino-3-methylimidazo(4,5-f)quinoline (IQ), could be detected. The mutagenic activity of 2-AA using BMX100 expressing CYP1A2 alone and in combination with rat CYP reductase was respectively 10 and 20 times higher than in BMX100 with the standard metabolic activation system, rat liver S9 fraction. Furthermore, the mutagenicity of 2-AA could be nullified by a-naphthoflavone, a known inhibitor of CYP1A2. IQ responded equally in BMX100 expressing the CYP1A2-reductase fusion protein as compared with usage of rat liver S9 fraction. Rat liver S9 fraction was much more potent in generating a mutagenic response to AFB1 in BMX100 than in the strain expressing human CYP1A2 alone or CYP1A2 fused to rat reductase. The results described in this study demonstrate that this new E. coli strain can function as a human CYP1A2-competent prokaryotic mutagenicity test system and they seem to characterize BMX100 as a strain of interest for studies to identify individual human CYPs involved in bioactivation and bioinactivation reactions of putative genotoxins.

Kulling SE, Jacobs E, Pfeiffer E, Metzler M. **Studies on the genotoxicity of the mammalian lignans enterolactone and enterodiol and their metabolic precursors at various endpoints in vitro.** Mutat Res 1998;416(1-2):115-24.
BIOSIS COPYRIGHT: BIOL ABS. The mammalian lignans enterolactone (ENL) and enterodiol (END) are formed by

intestinal bacteria from the plant lignans matairesinol (MAT) and secoisolariciresinol (SEC), respectively, which are ingested with different types of food. ENL and END are weak estrogens. According to epidemiological and biochemical studies, lignans may act as anticarcinogens, but little is known about their genotoxic potential. We have therefore investigated the effects of ENL, END, MAT and SEC on cell-free microtubule assembly and at the following genetic endpoints in cultured male Chinese hamster V79 cells: disruption of the cytoplasmic microtubule complex, induction of mitotic arrest, induction of micronuclei and their characterization by CREST staining, and mutagenicity at the HPRT gene locus. The lignans were tested at concentrations of 200 muM in the cell-free system and 100 muM in cultured cells, which represents the limit of solubility in each assay. The established aneuploidogen diethylstilbestrol and the clastogen 4-nitroquinoline-N-oxide were used as positive reference compounds. As none of the four lignans had any activity at the endpoints studied, we conclude that ENL, END, MAT and SEC are devoid of aneuploidogenic and clastogenic potential under the experimental conditions used in this study.

Lebailly P, Vigreux C, Godard T, Sichel F, Bar E, Letalaer JY, Henry-Amar M, Gauduchon P. **Assessment of DNA damage induced in vitro by etoposide and two fungicides (Carbendazim and Chlorothalonil) in human lymphocytes with the comet assay.** Mutat Res 1997;375(2):205-17.
The single cell gel electrophoresis (SCGE) assay was used to study the capacity of carbendazim (10605217) and chlorothalonil (1897456) alone or in association to induce DNA damage in human peripheral blood lymphocytes; the antitumor agent etoposide was used as a positive control. Lymphocytes were prepared from blood samples obtained from a healthy man, 23 years of age. Cells were exposed to the substance for 1 hour at 37 degrees-C. Cells were evaluated using the SCGE assay immediately after treatment and after a 24 hour post treatment incubation. The findings indicated that etoposide induced significant dose dependent DNA damages for concentrations at which the loss of cell viability is low. Following the 24 hour recuperation period, all observed DNA damages had disappeared. With SCGE assay performed after a 1 hour treatment, similar positive results were obtained with chlorothalonil alone or in association with carbendazim, without any loss of cell viability. However, a dramatic loss of cell viability was noted after 24 hours and was associated with a large proportion of highly damaged cells. Carbendazim was not cytotoxic on human peripheral blood lymphocytes and did not induce DNA damage using the SCGE assay either immediately after treatment or after a 24 hour post treatment incubation. The authors suggest that these findings indicate the need for an adequate evaluation of immediate and long term cytotoxicity of compounds that are to be assessed by the SCGE assay.

Lehmann J, Pollet D, Peker S, Steinkraus V, Hoppe U. **Kinetics of DNA strand breaks and protection by antioxidants in UVA- or UVB-irradiated HaCaT keratinocytes using the single cell gel electrophoresis assay.** Mutat Res 1998;407(2):97-108.
The aim of this study was to characterize the genotoxic action of UVA and UVB in human keratinocytes by application of the single cell gel electrophoresis assay (SCGE assay). Dose dependence of DNA damage, the time course of its repair, and the influence of cellular antioxidant status were assessed. Irradiation with UVA or UVB both resulted in a dose-dependent increase in the level of DNA damage. A time course study to evaluate the repair kinetics in keratinocytes irradiated with 5 J/cm2 UVA revealed an immediate occurrence of DNA effects which subsequently disappeared within about 1 h, indicating removal of DNA lesions. This rapid repair of DNA damage is consistent with the observation that 5 J/cm2 UVA did not impair cellular viability. In contrast, exposure to 15 mJ/cm2 UVB resulted in a prolonged repair of DNA damage which lasted about 25 h. Thus, the repair kinetics of UVA- and UVB-induced DNA damage clearly differed from each other, implicating the induction of different types of DNA lesions by UVA and UVB. Neither a pretreatment with Mg-ascorbyl phosphate or D,L-alpha-tocopherol, nor depletion of endogenous glutathione altered cellular sensitivity to UVB. In contrast, the DNA damaging effects of UVA could be counteracted by a pretreatment with these antioxidants. These observations confirm that the UVA-induced effects on DNA are related to radical mediated strand breaks and DNA lesions forming alkali-labile sites. The UVB-induced effects mainly occur as a consequence of excision repair-related strand breaks. The observed repair kinetics of DNA lesions and the influence of cellular antioxidant status may help to elucidate protective mechanisms against the carcinogenic effects of UV radiation present in sunlight.

Li Huaixing, Yang H, Li Jianxiu, Hu Yiping, Wang Xiaopeng, Hao Guangrong, Fu Jiliang. **EMS-induced mutant**

frequency and spectrum in bone marrow of D6-2 transgenic mice. Sci China C Life Sci 1998;41(3):286-92.
BIOSIS COPYRIGHT: BIOL ABS. EMS-induced mutant frequency and mutation spectrum as well as background mutant frequency have been characterized for bone marrow of the D6-2 transgenic mice. The lacI genes carried on pSPORT1 vectors were recovered from the treated or untreated mouse genomic DNA by excision and circularization, and analyzed in vitro for mutations that occurred in the mouse bone marrow. lacI- mutants were positively selected with the M9/L media. The 6 lacI- mutants were identified out of 11 935 vectors recovered from genomic DNA of the treated mice (mutant frequency was 50n 11 649 vectors from untreated mice (the background mutant frequency was lower than 8.6 mutant, in which the majority of sensitive sites for inactivation of the lacI gene product have been located, were sequenced and 16 mutation events were identified. The predominant mutations (14/16 or 87.5%) were base substitutions, whereas the remaining 2 mutations were single base deletions (12.5%). Of these base substitutions, transversions made up 9/14 or 64%, and transitions comprised 5/14 or 36%. These findings were markedly different from the spontaneous spectra characterized by using Big-Blue' system, as well as from the EMS-induced mutation spectra obtained with in vitro assay systems, where the EMS-induced predominant mutations are GC-AT transitions. In addition, 45% of mutations analyzed occurred at CpG dinucleotides, which was in accordance with previous studies with other systems. These data show that: (i) the D6-2 transgenic mouse lineage is a suitable model for studying mutagenesis in vivo; (ii) a fundamental difference in mutagenesis for EMS between in vitro and in vivo assay systems may exist, but more extensive sequence analyses are required to determine the possible differences in mutation spectra.

Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, Brookman KW, Siciliano MJ, Walter CA, Fan W, et al. **XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages.** Mol Cell 1998;1(6):783-93.
The phenotypically similar hamster mutants irs1 and irs1SF exhibit high spontaneous chromosome instability and broad-spectrum mutagen sensitivity, including extreme sensitivity to DNA cross-linking agents. The human XRCC2 and XRCC3 genes, which functionally complement irs1 and irs1SF, respectively, were previously mapped in somatic cell hybrids. Characterization of these genes and sequence alignments reveal that XRCC2 and XRCC3 are members of an emerging family of Rad51-related proteins that likely participate in homologous recombination to maintain chromosome stability and repair DNA damage. XRCC3 is shown to interact directly with HsRad51, and like Rad55 and Rad57 in yeast, may cooperate with HsRad51 during recombinational repair. Analysis of the XRCC2 mutation in irs1 implies that XRCC2's function is not essential for viability in cultured hamster cells.

Lu PJ, Ho IC, Lee TC. **Induction of sister chromatid exchanges and micronuclei by titanium dioxide in Chinese hamster ovary-K1 cells.** Mutat Res 1998;414(1-3):15-20.
BIOSIS COPYRIGHT: BIOL ABS. Titanium dioxide (TiO2) has color properties of extreme whiteness and brightness, is relatively inexpensive, and is extensively used as a white pigment in a variety of materials. TiO2, an effective blocker of ultraviolet light, is frequently added to sunscreens and cosmetic creams. However, the genotoxicity of TiO2 remains to be controversial. In this report, we have demonstrated that TiO2 can be transported into Chinese hamster ovary-K1 (CHO-K1) cells. The effects of TiO2 on induction of sister chromatid exchanges (SCE) and micronuclei (MN) were then studied in these cells. The SCE frequency in CHO-K1 cells treated with TiO2 at a nonlethal dose range (0 to 5 muM) for 24 h was significantly and dose-dependently increased. By the conventional MN assay, TiO2 at the dose ranged from 0 to 20 muM slightly increased the MN frequency in CHO-K1 cells. However, in the cytokinesis-block MN assay, the number of MN per 1000 binucleated cells was significantly and dose-dependently enhanced in CHO-K1 cells treated TiO2 at the same dose range for 24 h. These results suggest that TiO2 is a potential genotoxic agent.

Lynn S, Lai HT, Kao SM, Lai J, Jan KY. **Cadmium inhibits DNA strand break rejoining in methyl methanesulfonate-treated CHO-K1 cells.** Toxicol Appl Pharmacol 1997;144(1):171-6.
The effects of cadmium (7440439) on DNA excision repair were examined. Chinese-hamster-ovary (CHO) K1 cells were incubated with 0 to 2.0 micromolar (microM) cadmium-chloride (10108642) for 10 hours (hr) or 0.6 millimolar (mM) methylmethanesulfonate (66273) (MMS) for 1hr. Some MMS treated cells were then washed and incubated in medium containing 0 to 2.0microM cadmium-chloride for 10hr. The cells were analyzed for DNA strand breaks

(SBs) using the alkaline comet assay. MMS induced a high level of DNA SBs. Cadmium at 0.25 to 2.0microM induced only a small amount of DNA SBs. The level of MMS induced DNA damage decreased during 10hr incubation in MMS free medium. The repair of DNA SBs was retarded if cadmium was present in the medium, the effect being dose dependent. CHO K1 cells were incubated with 0 or 0.4mM MMS for 1hr, 0 to 2.0microM cadmium-chloride for 4hr, or 10mM hydroxyurea (HU) plus 100microM cytosine-beta-D-arabinofuranoside (araC) for 1 to 4hr. The cells were then incubated for 10hr in MMS free medium in the presence or absence of 0.25 to 2.0microM cadmium-chloride or 10mM HU plus 100microM araC. HU plus araC induced only a small amount of DNA breakage, but potentiated the DNA breaks induced by MMS. Cadmium when present during HU plus araC treatment did not decrease the level of DNA SBs. Cadmium significantly inhibited repair of these SBs. CHO K1 cells were dialyzed to produce a cell free extract that was known to contain proteins needed for DNA repair. To the extract was added 400 nanograms of MMS treated plasmid DNA or a polynucleotide composed of poly (deoxyguanosine)/poly(deoxythymidine). Up to 500microM cadmium-chloride was added. The effects of cadmium on repair of the SBs in plasmid DNA, DNA-polymerase activity, and the extent of DNA ligation of the polynucleotide were determined. Cadmium inhibited DNA repair, DNA-polymerase, and DNA ligation in a concentration dependent manner, the median inhibitory concentrations being 55, 26, and 10microM, respectively. The authors conclude that the mechanism of cadmium inhibition of DNA SB repair may be explained, at least in part, by cadmium binding to proteins involved in DNA ligation.

Maier A, Nebert DW. **Aromatic hydrocarbon receptor polymorphism: development of new methods to correlate genotype with phenotype.** Environ Health Perspect 1998;106(7):421-6.
Differential CYP1A1 inducibility, reflecting variations in aromatic hydrocarbon receptor (AHR) affinity among inbred mouse strains, is an important determinant of environmental toxicity. We took advantage of the Ahr polymorphism in C57BL/6 and DBA/2 mice to develop an oligonucleotide-hybridization screening approach for the rapid identification of DNA sequence differences between Ahr alleles. Oligonucleotides containing single-base changes at polymorphic sites were immobilized on a solid support and hybridized with C57BL/6 or DBA/2 AHR cDNA radiolabeled probes. The observed hybridization patterns demonstrate that this approach can be used to detect nucleotide differences in the Ahr coding region with very high accuracy. In parallel experiments, we used a yeast two-hybrid system to assess phenotypic differences in AHR function. AHR activation, as measured by ss-galactosidase reporter activity in Saccharomyces cerevisiae strain SFY526, was determined following treatment with varying doses of the AHR ligand ss-naphthoflavone (BNF). We found that the C57BL/6 AHR has about a 15-fold higher affinity for BNF than the DBA/2 AHR, in much better agreement with results reported for whole-animal studies than the values observed by in vitro ligand-binding assays. Using C57BL/6 and DBA/2 AHR chimeric proteins, we also confirmed the previously reported observation that an A375V change is principally responsible for the high- to low-affinity AHR phenotype. There has been no straightforward method to reliably and reproducibly phenotype large numbers of humans for CYP1A1 inducibility or AHR affinity. Screening human AHR cDNAs by oligonucleotide-hybridization and yeast two-hybrid methodologies will be invaluable for the rapid and unequivocal determination of changes in DNA sequence and receptor-ligand affinities associated with human AHR polymorphisms.

Mateu MG, Escarmis C, Domingo E. **Mutational analysis of discontinuous epitopes of foot-and-mouth disease virus using an unprocessed capsid protomer precursor.** Virus Res 1998;53(1):27-37.
An unprocessed capsid precursor (P1) of foot-and-mouth disease virus (FMDV) has been expressed in mammalian cells to study discontinuous epitopes involved in viral neutralization. Amino acid replacements found in virus-escape mutants were engineered in the P1 precursor by site-directed mutagenesis of the plasmid. In all cases the replacements abolished recognition of unprocessed P1 by the relevant monoclonal antibodies (MAbs), paralleling the effects of the corresponding substitutions in neutralization of infectious FMDV. Five capsid surface residues within the same discontinuous antigenic area that were never found replaced in escape mutants were also engineered in P1. None of the substitutions affected antibody recognition, suggesting that these residues were not directly involved in the interaction with the antibodies tested. The results validate site-directed mutagenesis of constructs encoding capsid precursors as an approach to probe the structure of viral discontinuous epitopes not amenable to analysis with synthetic peptides.

McGregor WG, Wei D, Chen RH, Maher VM, McCormic JJ. **Relationship between Adduct Formation, Rates of Excision Repair and the cytotoxic and Mutagenic Effects of Structurally-Related Polycyclic aromatic Carcinogens.** Mutat Res 1997;376(1-2):143-52.
The cytotoxic and mutagenic effect of (+-)-7beta,8alpha-dihydroxy-9alpha,10-epoxy-7,8,9,10-tetrahydrobe- nzo(a) pyrene (60268851) (BPDE) and those of two other polycyclic aromatic carcinogens, 1-nitrosopyrene (86674513) (1-NOP) and N-acetoxy-2-acetylaminofluorene (6098448) (N-AcO-AAF) were investigated using repair proficient diploid human fibroblasts. The BPDE induced adducts were significantly more cytotoxic and mutagenic than 1-NOP or N-AcO-AAF induced adducts, but the 1-NOP induced adducts were repaired two to three times faster than BPDE induced adducts and much faster than N-AcO-AAF induced adducts. BPDE induced adducts formed on the N2 position of guanine, whereas 1-NOP and N-AcO-AAF induced adducts formed at the C8 position of guanine. BPDE induced adducts were much more cytotoxic and mutagenic than adducts induced by the other two carcinogens. As the rate of repair of adducts induced by 1-NOP was faster, this may contribute to their lower mutagenic potential. The data on site specific rates of repair suggested that the interaction between the specific adduct conformation and the local DNA conformation at a specific nucleotide site influences the rate of repair at that site. The findings indicated that the cytotoxicity and mutagenicity of these polycyclic aromatic carcinogens in diploid human fibroblasts is determined by a complex interplay of adduct conformation, ability of adducts to block DNA replication and transcription, and variation in the rate of excision, even at the nucleotide level.

Mersch-Sundermann V, Kevekordes S, Jenter C. **Testing of SOS induction of artificial polycyclic musk fragrances in E. coli PQ37 (SOS chromotest).** Toxicol Lett 1998;95(3):147-54.
Synthetic fragrances are widespread in the environment. Residues were found in animals, human tissues and breast milk. Therefore, six artificial polycyclic musk fragrances--Galaxolide, Tonalide, Celestolide, Phantolide, Cashmeran and Traseolide--were tested for SOS induction using the Escherichia coli PQ37 genotoxicity assay (SOS chromotest) in the presence (+S9) and absence (-S9) of an exogenous metabolizing system. All compounds tested exhibited no SOS inducing potency with the SOS chromotest. These results could be rated as one indicator of the biological inactivity of this group of compounds with respect to genotoxicity.

Miyamae Y, Zaizen K, Ohara K, Mine Y, Sasaki YF. **Detection of DNA lesions induced by chemical mutagens by the single cell electrophoresis (Comet) assay. 1. Relationship between the onset of DNA damage and the characteristics of mutagens [corrected and republished article originally printed in Mutat Res 1997 Sep 18;393(1-2):99-106].** Mutat Res 1998;415(3):229-35.
We evaluated the relationship between the onset of DNA damage and the characteristics of 5 model chemical mutagens with the single-cell gel electrophoresis (SCG) assay using L5178Y mouse lymphoma cells. We treated the cells with each chemical for 3 h and sampled them 0.21, and 45 h after treatment. DNA damage induced by UV mimetic mutagens MMS and MNU, and X-ray mimetic mutagen BLM was observed just after treatment, crosslinking agent MMC-induced DNA damage was detected 21 h after treatment, and 6-MP as an inhibitor of DNA synthesis did not induce DNA damage at any sampling time. These results suggest that the SCG assay detects DNA lesions just after treatment with UV and X-ray mimetic mutagens, but needs a waiting period after treatment with crosslinking agents.

Miyata Y, Saeki KI, Kawazoe Y, Hayashi M, Sofuni T, Suzuki T. **Antimutagenic structural modification of quinoline assessed by an in vivo mutagenesis assay using lacZ-transgenic mice.** Mutat Res 1998;414(1-3):165-9.
BIOSIS COPYRIGHT: BIOL ABS. Quinoline, a hepatocarcinogen, mutates the bacterial tester strains in the presence of the rat liver microsomal enzymes and induces GST-P (placental glutathione S-transferase)-positive foci in a medium-term bioassay system for hepatocarcinogenesis. On the other hand, 3-fluorinated quinoline was neither mutagenic nor carcinogenic in the same assay systems, whereas, 5-fluoroquinoline was mutagenic and carcinogenic. Quinoline was recently demonstrated to be mutagenic in an in vivo mutagenicity assay system using the lacZ-transgenic mouse (Muta Mouse). The present study was undertaken to know whether 3-fluoroquinoline would be devoid of in vivo mutagenicity in Muta Mouse. Quinoline and 5-fluoroquinoline were also tested in the same system. Mutagenicity was evaluated in the liver, the target organ of quinoline carcinogenesis, and also in the

bone marrow and testis. The results strongly indicate that fluorine-substitution at the position-3 of quinoline could be an anti-genotoxic structural modification of quinoline in a wide range of its genotoxic end-points.

Moore MM, Harrington-Brock K, Doerr CL. **Relative genotoxic potency of arsenic and its methylated metabolites.** Mutat Res 1997;386(3):279-90.
The relative genotoxic potency of inorganic and organic arsenicals was studied in-vitro. Cultures of the TK(+/-) heterozygote of L5178 mouse lymphoma cells were incubated with up to 2.0 micrograms per milliliter (microg/ml) sodium-arsenite (7784465), up to 14.0microg/ml sodium-arsenate (7631892), up to 10,000microg/ml dimethylarsinic-acid (124652) (DMA), or up to 4,500microg/ml monomethylarsonic-acid (2163806) (MMA) for 4 hours. Cytotoxicity was assessed by measuring decreases in survival as determined from decreases in colony forming ability. Genotoxicity was evaluated by determining the frequency of mutations at the TK(+/-) locus, chromosome aberrations, m