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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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. **One-upping the LD50 [news]**. Environ Health Perspect 1998;106(10):478.

Boyd K. **Bringing both sides together.** Camb Q Healthc Ethics 1999;8(1):43-5.

Bucher JR, Lucier G. **Current approaches toward chemical mixture studies at the National Institute of Environmental Health Sciences and the U.S. National Toxicology Program.** Environ Health Perspect 1998;106(Suppl 6):1295-8.
The National Institute of Environmental Health Sciences (NIEHS) has several new initiatives involving chemical mixtures and has recognized the need to develop new experimental approaches to enhance our efforts in this area. Responding to recent increases in nominations of complex occupational exposures for toxicologic assessment by the U.S. National Toxicology Program, the NIEHS and the National Institute for Occupational Safety and Health have begun a program to characterize exposures through field studies, identify biomarkers of exposure in workers, and recreate relevant mixed exposures in a laboratory setting. A second initiative with the National Center for Environmental Health/Centers for Disease Control and Prevention will examine blood samples from the U.S. National Health and Nutrition Examination Survey population surveys for selected endocrine-disrupting agents and for common patterns of persistent xenobiotics, providing critical information for the design of animal studies to assess risks of relevant chemical mixtures to humans. New toxicology testing methods (lower cost, faster) will enhance our ability to study chemical mixtures (e.g., dioxin and dioxinlike chemicals, combination AIDS therapies). Ongoing method development efforts involve in vitro functional toxicology assays, screens for estrogenic activity, and carcinogenesis studies in transgenic mice. A major scientific initiative with mixtures involves studies of individual and mixtures of dioxin and dioxinlike chemicals to determine if toxic equivalence factors predict carcinogenic potency in traditional and transgenic bioassays. Complementing these studies is an increased emphasis on physiologically based pharmacokinetic modeling, an activity central to the proper interpretation of chemical mixture studies.

Garcia-Arribas O, Perez-Calvo M, Ribas B. **[Bioassay on diptera larvae to detect cadmium toxicity and other compounds]**. An Real Acad Farm 1998;64(3):499-512. (Spa)
BIOSIS COPYRIGHT: BIOL ABS. Insects are generally used to test the acute toxicity of pollutants in water, hence little scientifical data exist on its application on metal exposure and intoxication of metal and organic compounds in these animals. To realize the biotest on diptera larvae of Parasarcophaga argyrostoma, there are used in each experiment five or more different toxic concentrations of the compound, which is homogenized with beef meat in little plastic boxes and each one with ten larvae like the controls submitted to the same process. Its synchronic growth makes the repetitivity and the standard

deviation very reliable and useful for the quality assurance and linearity of the results. The bioassay is also an interesting alternative method for the substitution of laboratory mammals and this work should encourage and conduct to support new data to evaluate the hazard of poisoning for any compound in aquatic toxicology, water wastes, in mammals, wildlife animals, and in environmental pollution. In this work, the larvae exposed to Cd2+ ions (CdCl2) at different concentrations: 1, 25, 50, 75, 100 mug Cd/g diet, are weighed at the beginning and 24, 48 and 72 hours after the exposure. The weight of the larvae at different times and concentrations exposed to the compounds, give us the % of growth inhibition, and after several experiments reproducibility, linearity and sensitivity. The results suggest that the cadmium exposure of P. argyrostoma larvae shows the alteration of weight and % weight increase at the concentration between 10-75 mug Cd/g diet. The 50% weight inhibition during development is attained with the dose of 25 mug Cd2+/g diet at 72 hours. The non observed effect level (NOEL) per os is fixed to 10 mug Cd2+/g diet. The non observed adverse effects level (NOAEL) should be fixed also at 10mug Cd2+/g diet. The acute lethal dose is established at 400 mug Cd2+/g diet.

Hill RN, Stokes WS. **Validation and regulatory acceptance of alternatives.** Camb Q Healthc Ethics 1999;8(1):73-9.

Klotzko AJ, Singer P. **Learning from Henry Spira [editorial]**. Camb Q Healthc Ethics 1999;8(1):3-5.

Leclaire J, De Silva O. **Industry experience with alternative methods.** Toxicol Lett 1998;102-103:575-9.
L'OREAL has been using alternative methods for almost 30 years and this has led to their current widespread in-house use for evaluation of local effects in safety and efficacy. Alternative methods are used daily to assess eye and skin tolerance, phototoxicity, photoprotection, skin sensitization, percutaneous absorption and skin and hair care. For eye irritation, many years of in-house studies have enabled us to develop and to select the most reliable tests. New in vitro methods have also been developed to help to understand the ocular irritation mechanisms which underlie the irritative properties of new chemicals. In the field of skin irritation, L'OREAL's work has focused mainly on the wide possibilities offered by reconstructed human skins to evaluate the skin tolerance of cosmetics. Today we have managed to introduce Langerhans cells in reconstructed epidermis to develop an alternative to skin sensitization. Besides these in-house investigations either in research or in evaluation, our laboratories have contributed actively to multicentric studies to help the prevalidation/validation process in various fields. The alternative approach is now totally integrated into the safety evaluation strategy, and this allowed L'OREAL to totally ban animal testing on cosmetic products several years ago. In vitro alternatives are very powerful tools: they allow the study of fine mechanisms and the use of human cells. This overall 'in vitro' approach is a scientific, ethical and industrial breakthrough.

Mitsumori K. **A Japanese view on a global toxicology testing program before ICH1 and after ICH4.** Toxicol Lett 1998;102-103:557-60.
Many unharmonized points on the toxicological evaluation on pharmaceuticals have been solved by the discussion for 8 years in the ICH. The following issues are representative ones in which the Japanese view on a toxicology testing program after ICH4 is different from that before ICH1: (1) a new guideline and pre-mating treatment period in reproductive toxicity; (2) 9-month chronic studies for non-rodent

species; (3) a new guideline of toxicokinetics; and (4) selection of high dose levels and a new guideline using short-term alternative tests in carcinogenicity. Hazard evaluation of pharmaceuticals to man in toxicological studies in Japan before ICH1 has been conducted on the basis of the traditional toxicological evaluation. After ICH4, in line with the ICH strategy, Japan will adopt the analytical/ integrative toxicological evaluation taking into account the 'weight of evidence' approach, exposures of the test drug and species differences between experimental animals and humans. However, since there still remain many things that need to be clarified for the better understanding of the guidelines, Japan should take more flexible regulatory approaches for the evaluation.

Munro L. **From vilification to accommodation: making a common cause movement.** Camb Q Healthc Ethics 1999;8(1):46-57.

Singer P. **Henry Spira's search for common ground on animal testing.** Camb Q Healthc Ethics 1999;8(1):9-22.

Zucco F, Vignoli AL. **In vitro toxicology in Europe (1986-1997).** Toxicol In Vitro 1998;12(6):741-928.
BIOSIS COPYRIGHT: BIOL ABS. The main aim of this report is to provide the state-of-the-art of in vitro toxicology in Europe over the last 10 years and the most recent trends of development. In this context the more specific goals are: (a) to provide a record of the main reference points in each European country (possibly official organisations), with particular attention to the recently annexed countries and to the Eastern countries; (b) to set up a picture of activities and initiatives in order to ensure better co-operation and promotion of concerted action between the various partners for the development, validation and acceptance of in vitro methods; (c) to trace the future challenges. The report is organised in two parts. The first part focuses on analysis of the activities that have contributed to the development of this area in the last 10 years. The development of a critical interest towards alternative methods in toxicology arose mainly from the increasing attention to animal welfare shown by animal movements, the scientific community and interested industries. In Europe the key step has been the approval of the Directive on animal experiments and the recognition of the need of research to sustain the development of alternative methods, which is now a specific area (pre-normative research) in many EC Programmes for Research and Technical Development. This first part also describes the activities undertaken in this field in Europe, the United States and Japan. The second part of the report contains the results of data collection obtained through questionnaires addressed to all organisations involved in alternative testing. In this case, only the umbrella organisations are considered whenever possible, since the discipline has now reached a complex and dynamic status that does not allow us to take into consideration all the single groups working on in vitro pharmaco-toxicology. Furthermore, this second part also collects and analyses the available databases, the previous validation studies and the EU Framework Programmes, in order to give all relevant information available up to now on in vitro toxicology. This report should allow the end-users to: optimise the outcome of past experience; facilitate access to the field; address future investments. Finally, we present conclusions and recommendations that will give useful information to the European Commission for the Fifth Framework Programme implementation.

CARCINOGENESIS

Balachandran R, Ter Haar E, Yalowich JC, Welsh MJ, Grant SG, Day BW. **Induction of human breast cancer cell apoptosis from G2/M preceded by stimulation into the cell cycle by Z-1,1-dichloro-2,3-diphenylcyclopropane.** Biochem Pharmacol 1999;57(1):97-110.
We have shown previously that Z-1,1-dichloro-2,3-diphenylcyclopropane (a.k.a. Analog II, A(II)) inhibits human breast cancer cell proliferation regardless of estrogen receptor status or estrogen sensitivity, and that its cellular targets include microtubules. In the present study, we investigated the apoptosis-inducing effects of A(II). MCF-7, MCF-7/LY2, and MDA-MB-231 cells all showed nuclear fragmentation in response to 100 microM A(II) when stained with Hoechst 33342 and examined by fluorescence microscopy. Pulsed field gel electrophoretic analysis showed that each of the cell lines also developed specific high molecular weight DNA fragments: a low level of 1-2 Mb fragments appeared after 6 hr, while 30-50 kb fragments accumulated subsequently. At 24 hr of drug exposure, the majority of cells became nonadherent, and the 30-50 kb fragments were restricted to detached MCF-7 and MDA-MB-231 cells. Both adherent and detached MCF-7/LY2 cells exhibited these fragments. A previous study by single-color (propidium) flow cytometry demonstrated that A(II) blocks MDA-MB-231 cells in G2/M of the cell cycle. More refined analyses in the present study showed this same result for MDA-MB-231 cells, but MCF-7 and MCF-7/LY2 cells did not reveal apparent drug-induced cell cycle block. A(II) demonstrated growth inhibitory, cell cycle-perturbing, and hypodiploidy-inducing activity against other human breast carcinoma lines, i.e. BT-20, CAMA-1, and SKBR-3, but no such actions in the non-tumorigenic, "normal" human breast epithelial line MCF-10A. Bromodeoxyuridine labeling and two-color flow cytometric analysis, however, suggested that A(II) caused stimulation into S phase, and that G2/M was the phase of the cell cycle from which cells apoptosed. A(II) caused cell rounding, detachment from the growth matrix, and nuclear shrinkage and fragmentation in parallel with biochemical changes. Cycloheximide inhibited A(II)-induced cell death, indicating that its toxicity requires de novo protein synthesis.

Bartram HP, Draenert R, Dusel G, Richter F, Liebscher E, Christl SU, Scheppach W, Kasper H. **Effects of sodium selenite on deoxycholic acid-induced hyperproliferation of human colonic mucosa in short-term culture.** Cancer Epidemiol Biomarkers Prev 1998;7(12):1085-9.
It has been shown that in vitro incubation of human colonic biopsies with the secondary bile acid deoxycholic acid (DCA) leads to the hyperproliferation of colonic crypt cells with an expansion of the proliferative zone, which is regarded as a biomarker of increased cancer risk. Sodium selenite (SSE), on the other hand, has been implicated as a protective agent in experimental studies, but toxic effects were reported as well, depending on the dose of SSE. To elucidate the effects of SSE on human colonic mucosa, biopsies from endoscopically normal sigmoid colon tissue of 30 subjects were incubated with 5 microM DCA or a combination of 5 microM DCA and SSE in concentrations of 5, 10, 20, 50, 80, and 100 microM, respectively. Equimolar NaCl incubations served as a control. Proliferating cells were labeled by bromodeoxyuridine immunohistochemistry, and the labeling index (LI) was computed. In the experiments using 5, 10, and 20 microM SSE, the whole crypt LI was significantly lower after DCA + SSE incubation (0.136, 0.118, and 0.110, respectively) compared to that after incubation with DCA alone (0.172, 0.157, and 0.165, respectively; $P < 0.01$). The corresponding LIs during DCA + SSE incubation were comparable to the LIs obtained after NaCl incubation (average LI = 0.14). Contrary to

this finding, severe cell damage was observed in the biopsies that were incubated with the higher SSE concentrations of 50 microM and above. The antiproliferative effects of SSE may indicate a possible protective effect in the prevention of human colon cancer development. However, the observed toxic effects of higher SSE concentrations strongly suggest the need for additional studies before general recommendations for the use of SSE in colon cancer prevention can be made.

Bergqvist M, Brattstrom D, Stalberg M, Vaghef H, Brodin O, Hellman B. **Evaluation of radiation-induced DNA damage and DNA repair in human lung cancer cell lines with different radiosensitivity using alkaline and neutral single cell gel electrophoresis.** Cancer Lett 1998;133(1):9-18.
BIOSIS COPYRIGHT: BIOL ABS. Using the comet assay, radiation-induced DNA strand breaks were evaluated in human lung cancer cell fines with different radiosensitivity (U-1285, U-1906E, U-1752 and U-1810). Single strand breaks were more sensitive indicators of the radiation-induced damage than double strand breaks. However, there was no consistent pattern in the way the various cell lines responded to 1-5 Gy of gamma-irradiation and all cell lines showed a remarkably efficient DNA repair after 1 h. In a separate study of the repair kinetics of DNA double strand breaks, the radioresistant cell line U-1810 showed a more efficient initial strand rejoining than the radiosensitive cell line U-1285 after irradiation at 2 Gy. The latter finding suggests that the detection of early DNA repair may be useful when monitoring the intrinsic radiosensitivity of human lung cancer cells.

Boutonnat J, Muirhead KA, Barbier M, Mousseau M, Ronot X, Seigneurin D. **PKH26 probe in the study of the proliferation of chemoresistant leukemic sublines.** Anticancer Res 1998;18(6a):4243-51.
Proliferative status and multidrug resistance status are key predictors of therapeutic outcome in acute myeloid leukemia. Although classical methods for proliferative assessment such as tritiated thymidine or BrdUrd incorporation, are correlated with treatment outcome, they are time consuming and difficult to standardize. As an alternative, we have evaluated the use of a dye dilution method using PKH26 to determine rate and extent proliferation in drug sensitive and resistant cell lines. When cells labelled with this fluorescent membrane intercalating dye divide, each resulting daughter cell receives half of the dye. Using flow cytometric analysis, it is possible to estimate the number of cells having undergone different numbers of cell divisions. Four different questions were addressed in these studies: a) does PKH26 give stable and reproducible labelling? b) does labelling with PKH26 alter cellular proliferation characteristics? c) is PKH26 a substrate for PGP and MRP? d) does PKH26 labelling alter PGP expression and/or PGP activity? We found that PKH26 labelling is stable, reproducible and has no effect on cell proliferation. It does not modify PGP activity or expression, nor does it appear to be a substrate for PGP or MRP, since the rate of decrease in fluorescence intensity is similar for sensitive and resistant cells which are proliferating at the same rate. Using the dye dilution method, it is possible to simultaneously assess PGP, proliferative status, and level of PGP expression. We conclude that the methods developed here provide a simpler, more complete means for assessment of the effects of the drug therapy on sensitive and resistant cell populations in patients with hematologic malignancies.

Calabrese EJ, Baldwin LA. **Can the concept of hormesis be generalized to carcinogenesis?** Regul Toxicol Pharmacol 1998;28(3):230-41.
The concept of hormesis (i.e., low-dose stimulation/high-dose inhibition) has been shown to be widely

generalizable with respect to chemical class, animal model, gender, and biological end point. The public health implication of this lack of linearity in the low-dose area of the dose-response curve raises the question of whether low doses of carcinogens will reduce cancer risk. Articles relating to the process of carcinogenesis (i.e., initiation, promotion, tumor development, and progression) were obtained from a recently developed chemical hormesis database and evaluated for their evidence of hormesis. Numerous examples in well-designed studies indicate that U- or J-shaped dose-response relationships exist with respect to various biomarkers of carcinogenesis in different animal models of both sexes. Examples of such J-shaped dose-response relationships in each stage of the process of carcinogenesis were selected for detailed toxicological examination. These results have important implications for both the hazard assessment of carcinogens and cancer risk assessment procedures. Copyright 1998 Academic Press.

Di Marco PN, Priestly BG, Buckett KJ. **Carcinogen risk assessment. Can we harmonise?** Toxicol Lett 1998;102-103:241-6.
Harmonisation of risk assessment (RA) is one of the priorities for sound chemical management set by Chapter 19, Agenda 21 of the United Nations Conference on the Environment and Development (UNCED) 1992 Earth Summit. The benefits of harmonisation are self evident and include transportability and consistency of RA outcomes, transparency and efficiency of process, and credible science. The outcomes of carcinogen RA are a description or classification of the carcinogenic hazard, the conditions under which cancers may be induced, and an estimate of a dose or exposure which poses a minimal, or otherwise defined, risk in exposed human populations. Weight-of-evidence based systems which classify carcinogenic hazards are part of, but do not substitute for, the risk assessment process. Carcinogen RA is based on assessment of appropriate toxicological and exposure data sets, which may have much in common. However, national policy frameworks can differ to the extent that RA outcomes may be quite different for the same chemical(s). Historically, differences in science policy have been greater for cancer RA compared to other toxic endpoints, with a tendency to differentiate cancer RA on the basis of presumed mechanism (i.e. genotoxic or non-genotoxic) and relevance to humans (some carcinogenic responses in animals may be considered not relevant for human RA). Significant strides towards harmonisation are being made, with reassessment of some national policies and participation in international harmonisation programmes, such as the ones being managed by the International Programme for Chemical Safety (IPCS). Alternative approaches to quantitative carcinogen RA are being considered which are more amenable to harmonisation, and one such approach being developed in Australia in connection with contaminated sites will be discussed.

Fu PP, Von Tungeln LS, Yi P, Xia Q, Kadlubar FF, Et al. **Neonatal mouse tumorigenicity bioassay**. Drug Inf J 1998;32(3):711-28.
IPA COPYRIGHT: ASHP The use of the newborn mouse bioassay as an alternative tumorigenicity assay is discussed, and data from mechanistic studies, including metabolism, DNA adduct formation, and ras-oncogene activation that aid in the interpretation of tumor experiments, are presented. Data suggest that this bioassay is very likely insensitive to some indirect-acting chemical carcinogens. Ongoing studies are focused on the sensitivity of this bioassay to indirect-acting carcinogens that are believed to exert their tumorigenicity through secondary mechanisms.

Fujita M, Tsuchida T, Fujita T, Higashino K. **Effects of quinolinone derivative, vesnarinone, in**

combination with irradiation on human lung cancer cell lines. Oncol Rep 1999;6(2):353-7.
The quinolinone derivative, vesnarinone, is a novel inotropic agent used in the treatment of heart failure. It has also been found that vesnarinone has a potential anti-cancer activity. To evaluate the anti-cancer activity of vesnarinone in combination with irradiation, we investigated the cytostatic and cytotoxic effects on human lung cancer cell lines (PC-9 and Lu 134A) using MTT assay and isobologram analysis. We also analyzed the nuclear fragmentation of tumor cells by flow cytometric analysis. Our study demonstrated that combination of vesnarinone with irradiation had an additive inhibitory effect on both PC-9 and Lu 134A tumor cell growth. Vesnarinone could improve the sensitivity of tumor cells to irradiation and thus the dose of irradiation could be reduced to half without decreased inhibitory effect. Significant increase of the tumor cell nuclear fragmentation was observed with combination of vesnarinone and irradiation. These results indicate that vesnarinone not only directly inhibited tumor cell growth, but also improved the sensitivity to irradiation. Combination of vesnarinone with irradiation may be an efficacious protocol for lung cancer treatment. The inhibitory effect was ascribed to inducing tumor cell apoptosis.

Goldstein LS, Weyand EH, Safe S, Steinberg M, Culp SJ, Gaylor DW, Beland FA, Rodriguez LV. **Tumors and DNA adducts in mice exposed to benzo[a]pyrene and coal tars: implications for risk assessment.** Environ Health Perspect 1998;106(Suppl 6):1325-30.
Current methods to estimate the quantitative cancer risk of complex mixtures of polycyclic aromatic hydrocarbons (PAH) such as coal tar assume that overall potency can be derived from knowledge of the concentration of a few carcinogenic components such as benzo[a]pyrene (B[a]P). Genotoxic damage, such as DNA adducts, is thought to be an essential aspect of PAH-induced tumorigenesis and could be a biomarker for exposure useful for estimating risk. However, the role of B[a]P and the relationship of adduct formation in tumorigenesis have not been tested rigorously in models appropriate for human health risk assessment. Therefore, we directly compared tumor induction and adduct formation by B[a]P and coal tars in several experimental protocols, including one broadly accepted and used by regulators. We found that B[a]P content did not account for tumor incidences after exposure to coal tars. DNA adducts were found in both tumors and tumor-free tissue and tumor outcomes were not predicted by either quantitation of total DNA adducts or by the DNA adduct formed by B[a]P. These data suggest that risk assessments based on B[a]P content may not predict accurately risk to human health posed by environmental PAH.

Gollapudi BB, Stott WT, Yano BL, Bus JS. **Mode of action considerations in the use of transgenic animals for mutagenicity and carcinogenicity evaluations.** Toxicol Lett 1998;102-103:479-84.
Genetically altered rodent models can be useful in facilitating the extrapolation of results from animal carcinogenicity studies to human risk assessment by contributing mode of action data. Transgenic mutation models make it possible to analyze mutations in vivo in any tissue of interest. Validation studies using genotoxic and epigenetic carcinogens indicated a good correlation between mutation induction and the tumor target tissues and have provided data on mode of tumorigenic action. However, carcinogenesis is a complex process and mutation induction in a given tissue does not always lead to tumors in that tissue. Genetically altered animal models such as the p53 +/- mouse can be useful in differentiating genotoxic carcinogens from those operating by non-genotoxic mechanisms. An understanding of the tumor responses of these short-term alternative transgenic and knockout mice to

epigenetic events such as tissue injury and enzyme induction at high maximum tolerated doses will eventually increase our level of confidence in these animal models for hazard evaluation and mechanistic studies.

Hussain SP, Harris CC. **Molecular epidemiology of human cancer.** Recent Results Cancer Res 1998;154:22-36.
A challenging goal of molecular epidemiology is to identify an individual's risk of cancer. Molecular epidemiology integrates molecular biology, in vitro and in vivo laboratory models, biochemistry and epidemiology to infer individual cancer risk. Molecular dosimetry of carcinogen exposure is an important facet of molecular epidemiology and cancer risk assessment. Carcinogen macromolecular adduct levels, cytogenetic alterations and somatic cell mutations can be measured to determine the biologically effective doses of carcinogens. Molecular epidemiology also explores host cancer susceptibilities, such as carcinogen metabolism, DNA repair, and epigenetic and genetic alterations in tumor suppressor genes. p53 is a prototype tumor suppressor gene and is well suited for analysis of mutational spectrum in human cancer. The analyses of germ line and somatic mutation spectra of the p53 tumor suppressor gene provide important clues for cancer risk assessment in molecular epidemiology. For example, characteristic p53 mutation spectra have been associated with: dietary aflatoxin B1 exposure and hepatocellular carcinoma; sunlight exposure and skin carcinoma; and cigarette smoking and lung cancer. The mutation spectrum also reveals those p53 mutants that provide cells with a selective clonal expansion advantage during the multistep process of carcinogenesis. The p53 gene encodes a multifunctional protein involved in the cellular response to stress including DNA damage and hypoxia. Certain p53 mutants lose tumor suppressor activity and gain oncogenic activity, which is one explanation for the commonality of p53 mutations in human cancer. Molecular epidemiological results can be evaluated for causation by inference of the Bradford-Hill criteria, i.e., strength of association (consistency, specificity and temporality) and biological plausibility, which utilizes the "weight of the evidence principle.".

Jones PA, Laird PW. **Cancer epigenetics comes of age.** Nat Genet 1999;21(2):163-7.
The discovery of numerous hypermethylated promoters of tumour-suppressor genes, along with a better understanding of gene-silencing mechanisms, has moved DNA methylation from obscurity to recognition as an alternative mechanism of tumour-suppressor inactivation in cancer. Epigenetic events can also facilitate genetic damage, as illustrated by the increased mutagenicity of 5-methylcytosine and the silencing of the MLH1 mismatch repair gene by DNA methylation in colorectal tumours. We review here current mechanistic understanding of the role of DNA methylation in malignant transformation, and suggest Knudson's two-hit hypothesis should now be expanded to include epigenetic mechanisms of gene inactivation.

Lee SK, Song L, Mata-Greenwood E, Kelloff GJ, Steele VE, Pezzuto JM. **Modulation of in vitro biomarkers of the carcinogenic process by chemopreventive agents.** Anticancer Res 1999;19(1a):35-44.
A structurally diverse group of chemopreventive agents was evaluated using in vitro biomarkers of the carcinogenesis process. With cultured human bronchial epithelial (BEAS-2B) cells, sulfur-containing compounds such as 1.2-dithiole-3-thione and sulforaphane, and phenolic compounds such as caffeic acid

phenethyl ester and genistein, showed potent inhibition of benzo(a)pyrene [B(a)P] metabolite-DNA binding. Phenolic compounds also demonstrated strong antioxidant activity. Most of the test compounds did not inhibit 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity with cultured mouse epidermal ME 308 cells, with the exception of sulfur-containing compounds, 1,2-dithiole-3-thione and sulforaphane, and a selenium compound, 1,4-phenylenebis (methylene)selenocyanate. With cultured Hepa 1c1c7 cells, sulforaphane and 1,2-dithiole-3-thione mediated strong induction of quinone reductase, and genistein and ursolic acid were moderate inducers. Chalcone, 1,4-phenylenebis (methylene)selenocyanate and caffeic acid phenethyl ester induced HL-60 cell differentiation. Interestingly, sulforaphane and caffeic acid phenethyl ester inhibited the total metabolism of benzo(a)pyrene with cultured BEAS-2B cells, and the distribution pattern of water-soluble metabolites was altered in comparison with the control groups. These data are suggestive of pleiotropic mechanisms that should prove beneficial when considering the chemopreventive activity of these substances. As a result, of the group of 25 agents tested, four were judged as superior cancer chemopreventive agents: caffeic acid phenethyl ester, 1,2-dithiole-3-thione, genistein, and sulforaphane.

Nair J, Barbin A, Velic I, Bartsch H. **Etheno DNA-base adducts from endogenous reactive species.** Mutat Res 1999;424(1-2):59-69.
Promutagenic etheno (varepsilon) adducts in DNA are generated through reactions of DNA bases with LPO products derived from endogenous sources or from exposure to several xenobiotics. The availability of sensitive methods has made it possible to detect three varepsilon-adducts in vivo, namely varepsilondA, varepsilondC and N2,3-varepsilondG. One probable endogenous source for the formation of these adducts arises from LPO products such as trans-4-hydroxy-2-nonenal (HNE), resulting in highly variable background varepsilon-adduct levels in tissues from unexposed humans and rodents. The range of background levels of varepsilondAx10-8dA detected inhuman tissues was <0.05 to 25 and in rodent tissues 0.02 to 10; the corresponding values for varepsilondCx10-8dC were 0.01 to 11 and 0.03 to 24, respectively. Part of this variability may be associated with different dietary intake of antioxidants and/or omega-6 PUFAs which oxidize readily to form 4-hydroxyalkenals, as varepsilondA and varepsilondC levels in WBC-DNA of female volunteers on a high omega-6 PUFA diet were drastically elevated. Increased levels of etheno adducts were also found in the liver of cancer-prone patients suffering from hereditary metal storage diseases, i.e., Wilson's disease (WD) and primary hemochromatosis (PH) as well as in Long-Evans Cinnamon rats, an animal model for WD. Increased metal-induced oxidative stress and LPO-derived varepsilon-adducts, along with other oxidative damage, may trigger this hereditary liver cancer. varepsilon-Adducts could hence be explored as biomarkers (i) to ascertain the role of LPO mediated DNA damage in human cancers associated with oxidative stress imposed by certain lifestyle patterns, chronic infections and inflammations, and (ii) to verify the reduction of these varepsilon-adducts by cancer chemopreventive agents. This article summarizes recent results on the formation, occurrence and possible role of varepsilon-DNA adducts in carcinogenesis and mutagenesis.

Oganesian A, Hendricks JD, Pereira CB, Orner GA, Bailey GS, Williams DE. **Potency of dietary indole-3-carbinol as a promoter of aflatoxin B1-initiated hepatocarcinogenesis: results from a 9000 animal tumor study.** Carcinogenesis 1999;20(3):453-8.
Indole-3-carbinol (I3C), a metabolite of glucobrassicin found in cruciferous vegetables, is documented

as acting as a modulator of carcinogenesis and, depending on timing and dose of administration, it may promote hepatocarcinogenesis in some animal models. In this study we demonstrate that, when given post-initiation, dietary I3C promotes aflatoxin B1 (AFB1)-induced hepatocarcinogenesis in the rainbow trout model at levels as low as 500 p.p.m. Trout embryos (approximately 9000) were initiated with 0, 25, 50, 100, 175 or 250 p.p.b. AFB1 by a 30 min immersion. Experimental diets containing 0, 250, 500, 750, 1000 or 1250 p.p.m. I3C were administered starting at 3 months and fish were sampled for liver tumors at 11-13 months. Promotion at the level of tumor incidence was statistically significant for all dietary levels, except 250 p.p.m. Relative potency for promotion markedly increased at dietary levels >750 p.p.m. We propose that more than one mechanism could be involved in promotion and that both estrogenic and Ah receptor-mediated pathways could be active. The estrogenicity of I3C, measured as its ability to induce vitellogenin (an estrogen biomarker in oviparous vertebrates) was evident at the lowest dietary level (250 p.p.m.), whereas CYPIA (a P450 isozyme induced through the Ah receptor pathway) was not induced until dietary levels of 1000 p.p.m. Therefore, at lower dietary levels, promotion by I3C in this model could be explained by estrogenic activities of I3C acid derivatives, as it is known that estrogens promote hepatocarcinogenesis in trout. Much stronger promotion was observed at high dietary I3C levels (1000 and 1250 p.p.m.), at which levels both CYP1A and vitellogenin were induced.

Spinardi AL, Kaneno R, Rodrigues MA, Salvadori DM, Rocha NS, Barbisan LF, Ribeiro LR, De Camargo JL. **Natural killer activity in a medium-term multi-organ bioassay for carcinogenesis.** Jpn J Cancer Res 1999;90(1):101-7.
Natural killer (NK) cell activity was evaluated after the initiation and promotion steps in a medium-term multi-organ bioassay for carcinogenesis. NK cell activity was assessed in vitro by Cr51 release assay at the 4th and 30th weeks of the experiment. Male Wistar rats were sequentially initiated with N-diethylnitrosamine (DEN i.p.), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN drinking water), N-methyl-N-nitrosourea (MNU i.p.), dihydroxy-di-N-propylnitrosamine (DHPN drinking water) and N,N'-dimethylhydrazine (DMH s.c.) at subcarcinogenic doses for 4 weeks (DMBDD initiation). One group was evaluated at the 4th week and the other was maintained without any further treatment until the 30th week. Two initiated groups were exposed through the diet to 2-acetylaminofluorene (2-AAF) or phenobarbital (PB), from the 6th until the 30th week. Five additional groups were studied to evaluate the effects of each initiator on NK activity. All groups submitted to initiation only, initiation plus promotion, or promotion only, developed significantly more preneoplastic lesions than the untreated control group. The main target organs for tumor development in the initiated animals were the liver and the colon, irrespective of treatment with 2-AAF or PB. NK cell activity was not affected by exposure to genotoxic carcinogens after initiation, at the 4th week. Treatments only with PB or 2-AAF did not change NK cell activity. However, decreased NK cell activity was registered in the group only initiated with DMBDD and in the group given DMBDD+2-AAF. This late depression of NK cell activity at the 30th week could be related to the production of suppressing molecules by the tumor cells.

Steele VE, Boone CW, Lubet RA, Crowell JA, Holmes CA, Sigman CC, Kelloff GJ. **Preclinical drug development paradigms for chemopreventives.** Hematol Oncol Clin North Am 1998;12(5):943-61.
Preclinical screening studies and animal efficacy testing models currently are used by the National Cancer Institute's chemoprevention drug discovery program to assess and identify chemical agents and

natural products that may have the potential to prevent human cancer. Identification of potential cancer preventing agents begins by subjecting each compound to a sequential series of short-term, in vitro prescreens of mechanistic, biochemical assays to provide quantitative data to help establish an early indication of chemopreventive efficacy and to assist in prioritizing agents for further evaluation in longer-term, in vitro transformation bioassays and whole animal models. Promising chemical agents or combinations of agents that work through different inhibitory mechanisms subsequently are tested in well-established, chemically induced, animal tumor models, which include models of the lung, bladder, mammaries, prostate, and skin. These preclinical bioassays afford a strategic framework for evaluating agents according to defined criteria, and not only provide evidence of agent efficacy, but also serve to generate valuable dose-response, toxicity, and pharmacokinetic data required prior to phase I clinical safety testing. Based on preclinical efficacy and toxicity screening studies, only the most successful agents considered to have potential as human chemopreventives progress into clinical chemoprevention trials.

Van Der Laan JW. **New perspectives for alternative approaches to carcinogenicity testing: a regulator viewpoint.** Toxicol Lett 1998;102-103:561-4.
Genotoxic compounds are thought more likely to be transspecies carcinogens inducing carcinomas in more than one species, implying a hazard to humans. Non-genotoxic compounds will have a certain threshold concentration below which they are definitely not carcinogenic. What are we looking for in the case of pharmaceuticals? New animal models would be helpful to enhance the quality of assessment of carcinogenic potential, based on 'weight of evidence', in order to reach the goal of replacing animal life span studies without compromising human safety. Such studies should supplement life span studies and provide additional information not readily available from the long-term assay. Short- or medium-term assays may be helpful in confirming the transspecies character of carcinogens.

Van Kreijl CF, Van Der Houven Van Oordt CW, Kroese ED, Sorensen IK, Breuer ML, Storer RD. **Evaluation of the Emu-pim-1 transgenic mouse model for short-term carcinogenicity testing [see comments]**. Toxicol Pathol 1998;26(6):750-6.
The value of the chronic rodent carcinogenicity assay in adequately predicting cancer risk in humans has become a matter of debate over the past few years. Therefore, more rapid and accurate alternative tests are urgently needed. Transgenic mouse models, those harboring genetic changes that are relevant to the multistage cancer process, may provide such alternative tests. Transgenic Emu-pim-1 mice, developed by Berns and coworkers in 1989, contain the pimn-1 oncogene, which is expressed at elevated levels in their lymphoid compartments. As a result, these mice are predisposed to the development of T-cell lymphomas. Because of the low incidence of spontaneous tumors and the increased sensitivity to N-ethyl-N-nitrosourea-induced carcinogenesis, Emu-pim-1 mice were suggested to be one of the first potential and attractive candidates to be used in short-term carcinogenicity testing. In the present article, the results from 2 recent short-term assays (with mitomycin C and x-rays) are briefly presented, together with a review of all 11 performed bioassays and their corresponding histopathologic and molecular data. The overall results allow the first evaluation of the Emu-pim-1 mouse model with regard to its usefulness in short-term carcinogenicity testing. It has been shown that the model is primarily suitable as a sensitive short-term assay for genotoxic carcinogens that not only induce (at least) gene mutations and/or large deletions and rearrangements but that also sufficiently target the lymphoid system. However, the

Emu-pim-1 mice lack sufficient sensitivity to justify their routine use in short-term carcinogenicity testing in general.

Van Steeg H, Klein H, Beems RB, Van Kreijl CF. **Use of DNA repair-deficient XPA transgenic mice in short-term carcinogenicity testing.** Toxicol Pathol 1998;26(6):742-9.
At present (putative) human carcinogens are identified via epidemiological studies and testing using the chronic 2-yr rodent bioassay. Both methods have severe limitations in that they are slow, insensitive, expensive, and are also hampered by many uncertainties. The development of methods to modify specific genes in the mammalian genome has provided promising new tools for use in identifying carcinogens and characterizing their (qualitative) risk. Several transgenic mouse lines are currently under study to test their possible use in short-term carcinogenicity testing. One such candidate alternative transgenic model is the XPA knock-out mouse. These mice have an almost complete deficiency in DNA nucleotide excision repair (NER). Nevertheless, XPA-deficient mice are viable and have a background of a low incidence of spontaneous development of cancers. Approximately 15% of the mice develop hepatocellular adenomas (only after 1.5 yr). After treatment with ultraviolet-B radiation or 7,12-dimethylbenz(a)anthracene, the XPA-deficient mice developed squamous cell carcinomas and papillomas, respectively, on their skin. Oral treatment of XPA-deficient mice with benzo [a]pyrene (B[a]P), 2-acetylaminofluorene (2-AAF), and 2-amino-1-methyl-6-phenylimidazo [4,5-b]-pyridine (PhIP) resulted in lymphomas (B[a]P), liver and bladder tumors (2-AAF), and intestinal adenomas plus lymphomas (PhIP). These results look encouraging, but it should be noted that the compounds and agents tested thus far have all been substrate for nucleotide excision repair. Animal studies with different genotoxic or nongenotoxic compounds, as organized for instance within the framework of the International Life Sciences Institute/Health and Environmental Sciences Institute program, are needed to further evaluate the suitability of the XPA model for short-term carcinogenicity testing.

Watson JM, Parrish EA, Rinehart CA. **Selective potentiation of gynecologic cancer cell growth in vitro by electromagnetic fields.** Gynecol Oncol 1998;71(1):64-71.
BIOSIS COPYRIGHT: BIOL ABS. Objective. Epidemiological data suggest that exposure to electromagnetic fields (EMF) may increase the risk of various cancers. We evaluated EMF effects on the in vitro growth response of human cell lines isolated from various reproductive tract tissues. We also assessed the effects of EMF on cisplatin- or paclitaxel-induced cytotoxicity. Methods. Endometrial, ovarian, and prostate cancer cell lines as well as immortalized endometrial stromal cells and immortalized ovarian epithelial cells were exposed continually to EMF. Proliferation was assessed by the metabolic activity assay, MTT, direct cell counting, and anchorage-independent colony formation in soft agar. Cytotoxicity induced by cisplatin or paclitaxel was assessed using the MTT assay. Results. Continuous exposure to EMF at field strengths of 2 G enhanced proliferation of two human prostate and three endometrial, but only one ovarian, cancer cell lines. EMF enhanced metabolic activity of cancer cells within 96 h and increased absolute cell number (anchorage-dependent proliferation) and colony forming efficiency (anchorage-independent proliferation) over sham-treated controls. EMF had no effect on cytotoxicity induced by the chemotherapeutic agents Taxol or cisplatin. Conclusions. Continuous exposure to EMF can enhance growth rates of transformed cells for some human epithelial cancers. Cancer cells from the steroid sex hormone regulated tissues of endometrium and prostate appeared to be

more responsive to EMF than cells from ovarian cancers.

Wolff JE, Pascha KP, Poppenborg H, Anderson R, Thulig CC, Krebs B. **Polyoxoanions are cytotoxic to malignant glioma cells.** Anticancer Drugs 1998;9(9):803-7.
We studied the cytotoxicity of five polyoxoanions on two human malignant glioma cell lines (T98G and 86HG39), a rat glioma cell line (C6) and a human fibroblast cell line (NIH-3T3) using MTT tests to measure the drug concentration killing 50% of the cells (LC50). Cisplatin was used as a reference agent. Cisplatin had the highest efficacy in three of the four cell lines. Only in T98G cells, one of the components (POA5) had a lower LC50 value (1.3 x 10(-6) mol/l) than cisplatin (2.5 x 10(-6)). POA5 was also the most cytotoxic polyoxoanion when the LC50 values of all four cell lines were averaged (6.6 x10(-6)). Average LC50 values of the other compounds were 10.9, 12.6, 19.0 and 19.2 x 10(-6) mol/l in POA1, POA2, POA3 and POA4, respectively. When the benign fibroblasts were used to calculate a therapeutic index as LC50 in fibroblasts divided by LC50 in glioma cells, POA5 was superior to cisplatin. These results indicate that polyoxoanions are cytotoxic for malignant glioma cells and that the most promising compound investigated here was POA5.

Yang RS, Thomas RS, Gustafson DL, Campain J, Benjamin SA, Verhaar HJ, Mumtaz MM. **Approaches to developing alternative and predictive toxicology based on PBPK/PD and QSAR modeling.** Environ Health Perspect 1998;106(Suppl 6):1385-93.
Systematic toxicity testing, using conventional toxicology methodologies, of single chemicals and chemical mixtures is highly impractical because of the immense numbers of chemicals and chemical mixtures involved and the limited scientific resources. Therefore, the development of unconventional, efficient, and predictive toxicology methods is imperative. Using carcinogenicity as an end point, we present approaches for developing predictive tools for toxicologic evaluation of chemicals and chemical mixtures relevant to environmental contamination. Central to the approaches presented is the integration of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) and quantitative structure--activity relationship (QSAR) modeling with focused mechanistically based experimental toxicology. In this development, molecular and cellular biomarkers critical to the carcinogenesis process are evaluated quantitatively between different chemicals and/or chemical mixtures. Examples presented include the integration of PBPK/PD and QSAR modeling with a time-course medium-term liver foci assay, molecular biology and cell proliferation studies. Fourier transform infrared spectroscopic analyses of DNA changes, and cancer modeling to assess and attempt to predict the carcinogenicity of the series of 12 chlorobenzene isomers. Also presented is an ongoing effort to develop and apply a similar approach to chemical mixtures using in vitro cell culture (Syrian hamster embryo cell transformation assay and human keratinocytes) methodologies and in vivo studies. The promise and pitfalls of these developments are elaborated. When successfully applied, these approaches may greatly reduce animal usage, personnel, resources, and time required to evaluate the carcinogenicity of chemicals and chemical mixtures.

CYTOTOXICITY

Argese E, Bettiol C, Giurin G, Miana P. **Quantitative structure-activity relationships for the toxicity of chlorophenols to mammalian submitochondrial particles.** Chemosphere 1999;38(10):2281-92.

The toxicity of a series of chlorophenols, determined by a short-term in vitro assay utilizing mammalian submitochondrial particles, was related to the physicochemical and structural properties of these compounds. Quantitative Structure-Activity Relationships were defined by correlating EC50 values with six molecular descriptors, chosen to represent lipophilic, electronic and steric effects: the n-octanol/ water partition coefficient (log Kow), the constant of Hammett (sigma sigma), the acid dissociation constant (pKa), the first order valence molecular connectivity index (1 chi v), the perimeter of the efficacious section (sigma D) and the melting point (m.p.). The results of regression analysis showed that log Kow is the most successful descriptor, indicating that the ability of chlorophenols to partition into the lipid bilayer of the mitochondrial membrane has an important role in determining their toxic effects. These results are consistent with a molecular mechanism of uncoupling action based on the chemiosmotic theory and on the protonophoric properties of chlorophenols. The quality of the QSAR models confirms the suitability of the SMP assay as a short-term prediction tool for aquatic toxicity of environmental pollutants acting on respiratory functions.

Beattie SH, Williams AG. **Detection of toxigenic strains of Bacillus cereus and other Bacillus spp. with an improved cytotoxicity assay.** Lett Appl Microbiol 1999;28(3):221-5.
An improved qualitative cell cytotoxicity assay for the detection of Bacillus cereus emetic and enterotoxin is described. The presence of toxin in culture supernatant fluids was detected by measurement with the tetrazolium salt MTT, as it adversely affects the metabolic status of cultured CHO cells. Psychrotrophic B. cereus isolates (65) were assessed for toxin production using the cytotoxicity assay, and 91% of culture supernatant fluids were cytotoxic. Toxin assessment using BCET-RPLA and ELISA immunoassays indicated that 51% and 85% of the cultures, respectively, were toxigenic. There were pronounced strain differences in the amount of toxin produced by the B. cereus isolates. Some isolates of B. circulans, B. laterosporus/cereus, B. lentus, B. licheniformis, B. mycoides, B. subtilis and B. thuringiensis were also toxigenic.

Bondy GS, Armstrong CL. **Cytotoxicity of nephrotoxic fungal toxins to kidney-derived LLC-PK1 and OK cell lines.** Cell Biol Toxicol 1998;14(5):323-32.
The nephrotoxic fungal toxins ochratoxin A (OA), ochratoxin B (OB) and citrinin (CIT) are natural contaminants of foods and feeds. While cytotoxicity assays have proven useful for establishing relative toxicity and structure function relationships within groups of fungal toxins, a drawback of in vitro bioassays is their susceptibility to variation depending on endpoint, target cell, and dosing strategy. These variables were explored for OA, OB, CIT using two continuous kidney cell lines (LLC-PK1 and OK) and four cytotoxicity assay endpoints. The nephrotoxic antibiotic gentamicin was used as a positive control for cytotoxicity throughout. In general, fungal toxin-induced cytotoxicity was more pronounced in LLC-PK1 cultures using mitochondrial dehydrogenase inhibition (MTT assay) as the endpoint. Altered dosing strategy, but not seeding density, consistently influenced cytotoxicity: CIT was more toxic to cells when added at the time of seeding, whereas OA was more toxic when added 24 h after cultures were seeded. Toxicity rankings for the fungal toxins were consistent with in vivo studies and were, in order of most to least toxic, OA > OB > CIT. The data indicate that LLC-PK1 and OK cells compare favorably to existing models in terms of sensitivity to nephrotoxic fungal toxins, but also that relatively minor changes in assay protocols can affect the cytotoxicity of individual toxins and comparative toxicity within a group of toxins.

Brauers A, Jung PK, Thissen H, Pfannschmidt O, Michaeli W, Hoecker H, Jakse G. **Biocompatibility, cell adhesion, and degradation of surface-modified biodegradable polymers designed for the upper urinary tract.** Tech Urol 1998;4(4):214-20.
OBJECTIVES: The aim of this study was to develop a short bioresorbable ureteric stent and to characterize polymers and their surface modifications with respect to biocompatibility, degradation kinetics, cell adhesion properties, and incorporation of biologically active substances. Poly(D,L-lactide) PDLLA, poly(D,L-lactide-co-glycolide) PDLLA-co-GLY, and poly(D,L-lactide-co-trimethylenecarbonate) PDLLA-co-TMC were chosen as basic polymers. Surface modification was performed by plasma-induced graft polymerization and included grafting with hydroxyethylmethacrylate (HEMA), oligo(ethyleneoxide)-monomethacrylate (OEOMA), and acrylic acid (AAC). Biocompatibility of the polymers was assessed in vitro applying parameters of cell morphology, proliferative activity, and cell adhesion. All polymers were biocompatible and exerted no toxic effect on urothelial cell lines and on primary human urothelial cell cultures. A markedly reduced cell adhesion could be achieved in polymers grafted with HEMA, OEOMA, and AAC. Our results indicate that surface modification of bioresorbable polymers by grafting with HEMA, OEOMA, or AAC is an efficient approach to improve surface properties with respect to biocompatibility and cell adhesion properties.

Chang YC, Huang FM, Cheng MH, Chou LS, Chou MY. **In vitro evaluation of the cytotoxicity and genotoxicity of root canal medicines on human pulp fibroblasts.** J Endod 1998;24(9):604-6.
An intracanal medicine is often required because microorganisms in the dentinal tubes may be difficult to eliminate completely by instrumentation. Phenolic compounds are widely used in dental treatment as sedatives for the dental pulp or as disinfectants for caries and the root canal. In this study, propidium iodide fluorescence and DNA precipitation assay were used to investigated the cytotoxic and genotoxic effects of camphorated phenol and camphorated parachlorophenol on cultured human pulp fibroblasts in vitro. Both medicines reduced the content of double-stranded polynucleic acid of fibroblasts over a 24-h culture period in a concentration-dependent manner. Camphorated parachlorophenol was more cytotoxic than camphorated phenol. But, both medicines did not cause genotoxicity on pulp cells. The advantage of these experimental methods are simplicity and rapidity. Furthermore, this experimental system may be useful for preliminary cytotoxicity and genotoxicity screening of various dental medicines in vitro.

Chauvel-Lebret DJ, Pellen-Mussi P, Auroy P, Bonnaure-Mallet M. **Evaluation of the in vitro biocompatibility of various elastomers.** Biomaterials 1999;20(3):291-9.
A previous study highlighted the superior shock absorption of silicone rubbers compared to other elastomers. We evaluated and compared the in vitro biocompatibility of silicone-based rubbers and propose them as an alternative to conventional products. We used the MTT colorimetric test to assess cell viability and flow cytometry to evaluate cell proliferation. Tests were conducted at 24 and 72 h. Changes in cell morphology were evaluated by scanning electron microscopy. Positive (polyurethane) and negative (polystyrene) toxicity controls were included. The number of viable cells was significantly higher on polystyrene than on polyurethane. A decrease in the total number of cells from 24 to 72 h compared to the negative control was correlated with a lower percentage of S-phase cells. The differences in cell viability noted between the samples and the polystyrene control mainly resulted from an initial lack of adhesion, which was confirmed by scanning electron microscopy. The biocompatibility

of the three silicone rubbers was comparable to the best of the three products currently being used. These results, combined with those of the previous study, indicate that silicone rubber could be considered for the manufacture of mouth guards.

Hjertstedt J, Hahn BL, Kos WL, Sohnle PG. **Comparison of fungal viability assays using Candida albicans yeast cells undergoing prolonged incubation in the absence of nutrients.** Mycoses 1998;41 (11-12):487-92.
Staining methods for determining fungal viability are usually assessed by comparisons with enumeration of colony-forming units (CFU) on solid media. The purpose of the present study was to compare viability as assessed by the acridine orange (AO) and MTT methods with the numbers of CFUs obtained for Candida albicans yeast cells undergoing prolonged incubation in distilled water. In initial assessments of the assays using various proportions of control and heat-killed C. albicans, the AO and MTT methods consistently indicated significantly higher values for viability than did CFU determinations. Experiments using organisms cultured overnight revealed that approximately 95% of the cells were capable of dividing at least once in a microscopic proliferation assay, whereas only 69% were capable of forming colonies. Parallel assays comparing AO uptake and MTT reduction gave excellent agreement with the microscopic proliferation assay, but not with CFU determinations. Using organisms undergoing prolonged incubations in distilled water, much lower viabilities were obtained with the CFU method at 7 and 10 days than with the microscopic proliferation assay or the two staining methods. These results indicate that the AO and MTT assays correlate well with the ability of C. albicans to divide at least once, but may not accurately indicate the percentage of organisms actually able to form colonies.

Williams JM, Boyd B, Nutikka A, Lingwood CA, Barnett Foster DE, Milford DV, Taylor CM. **A comparison of the effects of verocytotoxin-1 on primary human renal cell cultures.** Toxicol Lett 1999;105(1):47-57.
Infection with verocytotoxin-producing Escherichia coli causes haemolytic uraemic syndrome (HUS). Verocytotoxin-1 (VT1) is cytopathic to renal microvascular endothelial cells in culture, supporting the hypothesis that the vasculopathy of HUS is caused directly by the toxic action of VT1 on cells. We provide evidence that VT1 inhibits protein synthesis in primary cultures of glomerular epithelial cells (GE), cortical tubular epithelial cells (CTE) and mesangial cells (MC). Using 100 pg/ml of VT1 we saw a decrease in protein synthesis to 14.3+/-1.9% in vero cells (a primate cell line), 1.7+/-0.3% in GE, 0.9+/-0.4% in CTE and 74.8+/-1.3% in MC at 24 h. The human renal epithelial cells are at least as sensitive as vero cells to the protein synthesis inhibitory effects of VT1 if not more so. Cell viability decreased in all cultures as measured by MTT reduction, neutral red incorporation and lactate dehydrogenase release and followed the same pattern of susceptibility as for protein synthesis inhibition. However, unlike vero cells, death occurred without DNA fragmentation. Cell sensitivity was greatest in cells which bound more VT1.

Zange R, Li Y, Kissel T. **Biocompatibility testing of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to a central poly(ethylene oxide) B block under in vitro conditions using different L929 mouse fibroblast cell culture models**. J Controlled Release 1998 Dec;56:249-58.
IPA COPYRIGHT: ASHP Different assay methods, including extraction, direct contact, and indirect

contact, based on L929 fibroblasts were compared for studying the in vitro biocompatibility of ABA triblock copolymers consisting of poly(glycolic-co-L-lactic acid) (poly(L-lactic-co-glycolic acid)) A blocks attached to a central polyethylene glycol (poly(ethylene oxide)) B block; the in vitro cytotoxicity and degradation of ABA triblock copolymers were also studied. The results showed that extraction was the most sensitive assay method. Polymer composition and molecular weight influenced the degradation rate and cytotoxicity of ABA triblock copolymers.

DERMAL TOXICITY

Barratt MD, Langowski JJ. **Validation and subsequent development of the DEREK skin sensitization rulebase by analysis of the BgVV list of contact allergens.** J Chem Inf Comput Sci 1999;39(2):294-8.
The DEREK knowledge-based computer system contains a subset of approximately 50 rules describing chemical substructures (toxophores) responsible for skin sensitization. This rulebase, based originally on Unilever historical in-house guinea pig maximization test data, has been subject to extensive validation and is undergoing refinement as the next stage of its development. As part of an ongoing program of validation and testing, the predictive ability of the sensitization rule set has been assessed by processing the structures of the 84 chemical substances in the list of contact allergens issued by the BgVV (German Federal Institute for Health Protection of Consumers). This list of chemicals is important because the biological data for each of the chemicals have been carefully scrutinized and peer reviewed, a key consideration in an area of toxicology in which much unreliable and potentially misleading data have been published. The existing DEREK rulebase for skin sensitization identified toxophores for skin sensitization in the structures of 71 out of the 84 chemicals (85%). The exercise highlighted areas of chemistry where further development of the rulebase was required, either by extension of the scope of existing rules or by generation of new rules where a sound mechanistic rationale for the biological activity could be established. Chemicals likely to be acting as photoallergens were identified, and new rules for photoallergenicity have subsequently been written. At the end of the exercise, the refined rulebase was able to identify toxophores for skin sensitization for 82 of the 84 chemicals in the BgVV list.

Benassi L, Bertazzoni G, Seidenari S. **In vitro testing of tensides employing monolayer cultures: a comparison with results of patch tests on human volunteers.** Contact Dermatitis 1999;40(1):38-44.
Evaluation of the irritant potential of new products or ingredients prior to human testing is generally performed in vivo on animals. However, according to the 6th amendment and following updates of the European Community directive on cosmetic products (93/35/EEC), animal testing will be banned when suitable substitutes will be available. To know whether in vitro tests for assessment of skin irritancy provide results approaching human conditions, comparisons have to be made between data deriving from in vitro tests and skin response in humans. The aim of our study was to assess the validity of the monolayer culture system of normal human keratinocytes as a model for the evaluation of the irritant effects of detergents, by comparing in vitro cell culture data to in vivo acute skin irritancy effects of cocamidopropyl betaine (CAPB), an amphoteric compound, Tween 20 (TW20) (polysorbate 20) and Tween 80 (TW80) (polysorbate 80), representing nonionic compounds, applied to the skin of 24 healthy volunteers at a concentration similar to that employed in commercial products. As parameters for

cytotoxicity, cell proliferation, cell membrane integrity and cell metabolism were assessed by cell counts, thymidine incorporation, MTT conversion, and Neutral Red uptake. In order to increase the sensitivity of the in vivo evaluation, bioengineering methods for assessment of the effects of test products on the skin were employed. Whereas all 4 in vitro methods ranked the tensides according to their toxicity in the following order: CAPB>SLS>TW20>TW80, both in vivo methods agreed in identifying SLS as the most irritating substance. Moreover, as compared with the irritation potential on human skin, all 4 in vitro tests overestimated the toxicity of CAPB. This suggests that the keratinocyte monolayer cell culture technique cannot directly replace in vivo methods, and that data obtained by this method should be interpreted cautiously.

Bernstein IA, Vaughan FL. **Cultured keratinocytes in in vitro dermatotoxicological investigation: a review.** J Toxicol Environ Health B Crit Rev 1999;2(1):1-30.
The keratinocyte is responsible for the architecture of the epidermis, that portion of the skin that forms the environmental barrier necessary for survival. It also interacts with other cell types in the epidermis in response to various environmental influences. This cell type is used frequently for in vitro cutaneous toxicological investigations as an alternative to whole-animal studies. Several areas of cutaneous research using cultured keratinocytes are germane as regards the scope of this journal. The following areas of biomedical research were reviewed: (1) dermatotoxicology, including environmental chemicals, antiseptics, drugs, metals, and pesticides; (2) immunotoxicology, including inflammation and allergic dermatitis; (3) radiation, including ultraviolet and x-irradiation; and (4) the development of assays as alternatives to whole-animal testing. Due to the abundance of such investigations reported in the last 30 years, this review is limited mainly to reviewing reports published in this decade.

Bolgul Y, Hekimoglu S, Sahin-Erdemli I, Kas HS. **Evaluation of oxprenolol hydrochloride permeation through isolated human skin and pharmacodynamic effect in rats**. STP Pharma Sci 1998;8(3):197-201.
IPA COPYRIGHT: ASHP Free and crosslinked poliglusam (chitosan) films containing 2 or 3% oxprenolol hydrochloride were prepared for transdermal drug delivery; drug permeation through human skin was studied in vitro and pharmacodynamics were examined in rats after application onto abdominal skin. Drug permeation through isolated human skin followed Q vs t1/2 kinetics, which is specific for matrix systems. Drug permeation through skin was dependent on the drug concentration and crosslinking agent; the amount of drug that permeated through skin increased with a higher drug concentration and decreased with crosslinking. Free chitosan film containing 3% oxprenolol was pharmacodynamically active after application onto the skin of rats.

Chang SL, Banga AK. **Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions**. J Pharm Pharmacol 1998 Jun;50:635-40.
IPA COPYRIGHT: ASHP The transdermal iontophoretic delivery of hydrocortisone solubilized in an aqueous solution of hydroxypropyl-beta-cyclodextrin was compared with the chemical enhancement effects of cosolvent formulations in vitro. The passive permeation of hydrocortisone through skin was higher when delivered from propylene glycol than after solubilization in the cyclodextrin. However, the iontophoretic delivery of 1% hydrocortisone-9% cyclodextrin was higher than the amount delivered passively in 1% hydrocortisone-propylene glycol, even in the presence of an absorption enhancer.

Iontophoretic delivery of 1% drug with 3% or 15% cyclodextrin was lower than that of the 9% cyclodextrin solution. The results suggested that free drug rather than complexes was predominantly delivered iontophoretically through skin and the cyclodextrin complex served as a carrier to replenish depletion of the drug. Iontophoresis provided better enhancement of drug delivery than the chemical approach when just enough cyclodextrin was added to solubilize the drug.

Cowan FM, Broomfield CA, Smith WJ. **Sulfur mustard exposure enhances Fc receptor expression on human epidermal keratinocytes in cell culture: implications for toxicity and medical countermeasures.** Cell Biol Toxicol 1998;14(4):261-6.
BIOSIS COPYRIGHT: BIOL ABS. Sulfur mustard (HD) is a chemical warfare blister agent. The biochemical basis of HD-induced vesication is unknown, and no antidote currently exists. Basal epidermal cells are a major site of HD toxicity in vivo, with inflammation and HD-increased proteolytic activity implicated as factors that contribute to HD pathology. Fc receptors (FcR) bind to the Fc region of antibody to mediate many effector and regulatory functions that can influence inflammatory responses. FcR are found on all types of immune cells and are also expressed on the surface of human keratinocytes. Assay by fluorescent antibodies demonstrated significantly enhanced CD32 (FcRII) and CD16 (FcRIII) on human epidermal keratinocyte (HEK) cell cultures at 8 to 24 h after exposure to HD (50, 100 and 200 mumol/L). The enhanced CD32 was time- and concentration-dependent and agreed well with the time course of increased proteolysis and cutaneous pathology observed during HD vesication. HD-increased FcR on the surface of HEK might be a mechanism of vesication.

Diener W, Kayser D, Schlede E. **The dermal acute toxic class method: test procedures and biometric evaluations.** Arch Toxicol 1998;72(12):751-62.
A dermal acute toxic class (ATC) method is presented with the use of significantly fewer animals in comparison with the classical dermal 50% lethal dose (LD50) test. The principle of the dermal ATC method is based on the oral and the inhalation ATC method. The method was developed for three fixed starting doses. Depending on the dermal LD50, the slope, the classification system and the starting dose on average 40 to 90% fewer animals will be used in comparison to at least 30 animals with the dermal LD50 test. The method was biometrically evaluated by using the Probit model for dose-response relationships. At present, there are eight different international classification systems based on dermal LD50 values. The test procedures and the calculations of the classification probabilities demonstrate that the dermal ATC method is a reliable alternative to the dermal LD50 test with the use of significantly fewer animals. Classification probabilities are presented for all classification systems currently in use, and expected numbers of experimental and of moribund/dead animals are demonstrated for the system of chemicals in the European Union for all three starting doses. The conclusion is justified that, similarly to the inhalation ATC method, there is no need to validate the dermal ATC method with the use of experimental animals.

Draye JP, Delaey B, Van De Voorde A, Van Den Bulcke A, De Reu B, Schacht E. **In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films.** Biomaterials 1998;19 (18):1677-87.
The biosafety of a new hydrogel wound dressing material consisting of dextran dialdehyde cross-linked gelatin was evaluated (i) in vitro in cultures of dermal fibroblasts, epidermal keratinocytes, and

endothelial cells, three cell types which play a major role in the process of cutaneous wound healing, and (ii) in vivo by subcutaneous implantation studies in mice. The cytotoxicities of this hydrogel, two semi-occlusive polyurethane dressings (Tegaderm and OpSite), and a hydrocolloid dressing (DuoDERM) were compared by measuring cell survival with the tetrazolium salt reduction (MTT) assay after incubations of the wound dressing samples for up to 6 d, in the presence of--but not in direct contact with--the cells. In vitro, the degree of cytotoxicity of the new hydrogel was greater in keratinocyte cultures than in fibroblast and endothelial cell cultures, and increased upon longer incubation time. In keratinocyte cultures, the semi-occlusive polyurethane dressings, the hydrocolloid, and the hydrogel dressings induced low, high and acceptable degrees of cytotoxicity, respectively. The toxicity of the isolated hydrogel components was assessed in Balb MK keratinocyte cultures. In these cells, epidermal growth-factor-stimulated thymidine incorporation into DNA was higher in the presence of gelatin. By contrast, concentrations of dextran dialdehyde as low as 0.002% were found to significantly decrease thymidine incorporation ($P < 0.01$). Subcutaneous implantation studies in mice showed that in vivo the hydrogel was biocompatible since the foreign body reaction seen around the implanted hydrogel samples was moderate and became minimal upon increasing implantation time. These results indicate that dextran dialdehyde cross-linked gelatin hydrogels have an appropriate biocompatibility.

Fang JY, Sung KC, Lin HH, Fang CL. **Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies.** Int J Pharm 1999;178(1):83-92.
The objective of this study was to evaluate the in vitro and in vivo transdermal iontophoresis of various diclofenac sodium polymer formulations. The excised rat skin, human skin as well as cellulose membrane were used to examine the in vitro drug permeation whereas the microdialysis technique was used to monitor the drug concentration in vivo. Polymer solutions based on polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) binary system showed higher drug permeability than that of single polymer vehicle. The effect of formulations on drug permeation through cellulose membrane was quite different from those through rat skin and human skin, which can be explained by the different permeation pathways between them. It appeared to be a membrane-controlled mechanism but not the vehicle matrix-controlled mechanism for diclofenac hydrogels when using skin as the diffusion barrier. The recovery of diclofenac sodium in the in vivo microdialysis was approximately 80-90%, indicating this technique can be used in the intradermal drug monitoring. For all the polymer formulations tested, there was a good relationship between the in vitro and in vivo drug permeation. A synergistic effect on drug permeation was observed when transdermal iontophoresis combined with the pretreatment of cardamom oil as a permeation enhancer.

Frenz DA, Yoo H, Liu W. **Basilar papilla explants: a model to study hair cell regeneration-repair and protection.** Acta Otolaryngol (Stockh) 1998;118(5):651-9.
Explants of basilar papillae from 6-7 days posthatch chicks were cultured in growth medium for a period of 1-8 days. Hair cells were counted following staining of stereocilia bundles with FITC-phalloidin, and the percentage of hair cell survival was determined by comparison to control (i.e. uncultured) specimens. Hair cell integrity was evaluated by scanning electron microscopy. Although previous studies have utilized organotypic culture of the basilar papilla to assess cell proliferation and ototoxicity, viability and integrity of hair cells was documented for periods of up to only 2 3 days. Our results demonstrate substantive auditory hair cell viability for a period of 7 days in vitro. We describe a pattern of natural

hair cell loss in organotypic culture that progresses along a proximal-distal, abneural-neural gradient, mimicking the pattern of hair cell loss that occurs following ototoxic insult to the chick basilar papilla in vivo and the pattern we observed during a 48-h period of exposure of basilar papilla explants to an ototoxic dose of neomycin. Our results provide an important quantitative step for the use of organotypic culture of the chick basilar papilla as a purposeful model to investigate the process of hair cell regeneration-repair in the avian auditory system.

Goettsch W, Garssen J, De Gruijl F, Dortant P, Van Loveren H. **Methods for exposure of laboratory animals to ultraviolet radiation.** Lab Anim 1999;33(1):58-67.
BIOSIS COPYRIGHT: BIOL ABS. Two different sources of ultraviolet B (UVB) radiation, an electronically controlled UVB exposure unit, containing FS40 tubes, and a hand-held Kromayer lamp, were evaluated for actual irradiance in W/m2 and spectra (physical dosimetry and biological dosimetry (skin effects in rodents)). The technical studies of the FS40 sources demonstrated that the flux intensity of the lamps could be changed electronically, without affecting the spectrum. Thus it was possible to standardize UVB exposure electronically. The biologically effective doses of these sources were analysed in RIV-Tox Wistar rats and BALB/c mice. After low doses of UVB radiation, histopathological changes such as acanthosis, hyperkeratosis and dermal inflammation were observed in the skin without the presence of major side effects such as erythema and oedema. After higher doses of UVB radiation erythema and oedema were clearly visible. Quantitative studies showed that the minimal erythema dose, as a biological parameter, correlated well to the emission in J/m2. In addition, biological parameters such as acanthosis and inflammation in the skin correlated well to the actual exposure in J/m2 and were sensitive biomarkers for UVB-induced skin toxicity. Thus, in addition to minimal erythemal doses, acanthosis and inflammation may also be applied is biologically relevant doses for studies of the biological effects of UVB radiation.

Gore AV, Liang AC, Chien YW. **Comparative biomembrane permeation of tacrine using Yucatan minipigs and domestic pigs as the animal model**. J Pharm Sci 1998 Apr;87:441-7.
IPA COPYRIGHT: ASHP To assess the potential of absorptive mucosa and skin as routes for the systemic delivery of tacrine and to compare the suitability of Yucatan minipigs and domestic pigs as animal models for this purpose, the in vitro permeation of tacrine across 4 absorptive mucosa of the pigs, nasal, buccal, sublingual, and rectal, in comparison to that across intestinal segments of the pigs and the in vitro skin permeation of tacrine across human cadaver skin and the dorsal skin of the pigs were studied. The intrinsic permeabilities and diffusivity of tacrine across the 4 absorptive mucosa were not significantly different between species, but they were lower than that observed across the intestinal segments of both species. Of the 4 mucosa, nasal mucosa had the highest permeability, while buccal mucosa had the lowest. The permeation of tacrine through domestic pig skin was about 2-fold higher than that across Yucatan minipig skin. The intrinsic permeability and diffusivity of tacrine across human cadaver skin was comparable to that of domestic pig skin. Overall, the results indicated that the domestic pig was the better animal model.

Hermanns-Le T, Arrese JE, Goffin V, Pierard GE. **Podophyllotoxin-induced acantholysis and cytolysis in a skin equivalent model.** Eur J Morphol 1998;36(3):183-7.
Skin equivalent models are used for a wide variety of pharmacotoxicological trials. The present study

was performed to assess morphologically the effect of podophyllotoxin on human bioengineered skin. The untreated model exhibited many resemblances with the parent tissues, although the epidermal differentiation was slightly impaired at the ultrastructural level. The penetration of podophyllotoxin and its biological effects inside the model appeared largely increased compared to the clinical experience with the drug. Acantholysis and cytolytic changes were prominent mimicking the effect of cantharidin. The exaggerated response of many skin equivalents to various compounds shed some doubts on the validity of the model when it is used to show efficacy rather than toxicity. This might apply to claims of efficacy for cosmetic compounds. The effect of cosmetic additives cannot be validated by such approach alone.

Homey B, Von Schilling C, Blumel J, Schuppe HC, Ruzicka T, Ahr HJ, Lehmann P, Vohr HW. **An integrated model for the differentiation of chemical-induced allergic and irritant skin reactions.** Toxicol Appl Pharmacol 1998;153(1):83-94.
Contact and photocontact allergic as well as irritant and photoirritant skin reactions represent a major problem in clinical dermatology and during the development of new pharmaceuticals. Furthermore, there is a lack of in vitro and in vivo assays that provide a clear differentiation between allergic and irritant skin reactions. Here, we describe an integrated model to differentiate between chemical-induced allergic and irritant skin reactions by measuring objective and easy-to-determine parameters within both skin and skin-draining lymph nodes. Dose-response studies with standard contact and photocontact allergens as well as irritants and photoirritants revealed that irritants predominantly induced skin inflammation, which in turn stimulated draining lymph node cell proliferation. In contrast, the induction phase of contact or photocontact allergy was characterized by marginal skin inflammation, but a marked activation and proliferation of skin-draining lymph node cells. Therefore, a differentiation index (DI) was defined describing the relation between skin-draining lymph node cell activation (lymph node cell count index) and skin inflammation (ear swelling). A DI > 1 indicates an allergic reaction pattern whereas DI < 1 demonstrates an irritant potential of a chemical. Experiments with the contact allergen oxazolone, the photocontact allergen TCSA + UVA, the irritant croton oil, and the photoirritant 8-methoxypsoralen + UVA confirmed the predictive value of DI. Furthermore, flow cytometric analysis of lymph node-derived T- and B-cell subpopulations revealed that contact sensitizer, but not irritant, induced the expression of CD69 on the surface of I-A+ cells. In conclusion, further studies with a broad range of irritants and allergens will be required to confirm general applicability.

Hosoya O, Sano M, Wada Y, Seki T, Morimoto Y, Et AL. **Effect of several hydrophilic polymers on the permeation of morphine and salicylic acid through excised hairless rat skin**. Chem Pharm Bull 1998 May;46:882-5.
IPA COPYRIGHT: ASHP The effects of various hydrophilic polymers on the permeation of morphine hydrochloride and salicylic acid through hairless rat skin were studied in vitro. Anionic polymers decreased the skin permeation of morphine and cationic polymers increased permeation. Nonionic polymers caused little change in morphine permeation. Cationic polymers decreased the skin permeation of salicylic acid and anionic polymers increased permeation. These opposite results were probably caused by the change in escaping tendency of drugs from the vehicles. The partition of morphine was increased and decreased by polymers having identical and opposite charge to the drug, respectively. The low partition of the drugs to skin may have also been caused by low diffusion of drugs in the polymer

solutions. The drug release rate from hydrophilic polymer solutions decreased in the presence of polymers having opposite charge to the drugs. It was suggested that the drug-polymer interactions changed the drug partition to skin and the skin permeation of the drug.

Muller-Decker K, Heinzelmann T, Furstenberger G, Kecskes A, Lehmann WD, Marks F. **Arachidonic acid metabolism in primary irritant dermatitis produced by patch testing of human skin with surfactants.** Toxicol Appl Pharmacol 1998;153(1):59-67.
A clinical study was performed to determine the effects of patch testing human skin with four industrially used surfactants on erythema formation, transepidermal water loss, and the contents in suction blister fluids of primary proinflammatory mediators including arachidonic acid, eicosanoids, and IL-1 alpha, which were analyzed by quantitative gas chromatography/negative ion chemical ionization mass spectrometry and by an enzyme-immunoassay, respectively. Benzalkonium chloride (BKCI) and sodium lauryl sulfate (SLS) elicited erythema and caused increased transepidermal water loss, indicating a disturbance of the epidermal barrier. Triethanolamine (TEA) and Tween 80 did not evoke these gross symptoms of inflammation. Suction blister fluids collected after a 24-h application of BKCl, SLS, and Tween 80 contained significantly increased amounts of individual eicosanoids whereas TEA induced no response. The induced eicosanoid profile was characteristic for each compound, pointing to different cell types of skin to be involved in their production. The elevation of prostaglandin and LTB4 contents correlated with the induction of erythema and the impairment of the epidermal barrier as shown for BKCl and SLS and preceded the maximum of erythema formation. IL-1 alpha contents did not correlate with these gross symptoms of inflammation. The results of this in vivo study support those of a previous study using human keratinocytes in culture indicating the release of arachidonic acid and prostaglandins to be an early event involved in the interaction of keratinocytes with surfactants. Moreover, the in vivo data with human skin underscore the mechanistic relationship to the in vitro model and support the concept that arachidonic acid and eicosanoid release from keratinocytes can be used as a marker of primary skin irritation.

Orth DS, Widjaja J, Ly L, Cao N, Shapiro WB. **Stability and skin persistence of topical products: evaluating the effect of a hydroalcoholic hydroquinone vehicle**. Cosmet Toiletries 1998 Oct;113:51-6, 58-63.
IPA COPYRIGHT: ASHP To determine the stability and compatibility of a hydroalcoholic vehicle containing hydroquinone for cosmetic and OTC drugs, the active ingredients of 7 commercially available cosmetic and OTC drug products were added to a 2% hydroquinone vehicle and were evaluated for stability in vitro and for skin persistence on volunteers and were compared with the commercially available product. The demonstrated range of stability was 0-6 months. In addition, the functional ingredients generally penetrated the skin more quickly when applied as hydroquinone mixtures than when applied alone.

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.
Ethylcellulose-polyvinyl pyrrolidone films containing diltiazem hydrochloride and indomethacin were evaluated for their potential drug delivery at a controlled rate, using rat skin, to select a suitable

formulation for the development of transdermal drug delivery systems. The influence of film composition, initial drug concentration, and film thickness on the in vitro drug release rate as well as drug permeation through rat abdominal skin were studied. Drug release studies were carried out employing the paddle over disk method and drug permeation through full thickness of the rat abdominal skin was tested using a modified Franz diffusion cell fastened with O-ring. The drug content of the film decreased at an apparent first-order rate, whereas the quantity of drug released was proportional to the square root of time. The release rates of both drugs increased linearly with increasing drug concentration and polyvinyl pyrrolidone fraction in the film, but was found to be independent of film thickness. The increase in release rate may be due to leaching of hydrophilic fraction of the film former which resulted in the formation of pores. It was also observed that the release of drugs from the films followed a diffusion-controlled model at low drug concentrations. A burst effect was observed initially, however, at high drug loading levels. This may be due to rapid dissolution of the surface drug followed by the diffusion of drug through the polymer network in the film. The in vitro skin permeation profiles showed increased flux values with increase of initial drug concentration in the film and also with the concentration of polyvinyl pyrrolidone. From this study, it is concluded that the films composed of ethylcellulose:polyvinyl pyrrolidone:diltiazem hydrochloride (8:2:2) and ethylcellulose:polyvinyl pyrrolidone:indomethacin (8:2:3) should be selected for the development of transdermal drug delivery systems, using a suitable adhesive layer and backing membrane, for potential therapeutic use.

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 May;46:831-6.
IPA COPYRIGHT: ASHP The enhancing effects of N-dodecyl-2-pyrrolidone (lauryl pyrrolidone) on the percutaneous absorption of doxifluridine, fluorouracil (5-fluorouracil; 5-FU), tegafur, and carmofur were studied using an in vitro penetration technique and rat skin. Permeability coefficients were significantly correlated with partition coefficients in phosphate buffered isotonic sodium chloride (saline). This suggested that the nonpolar stratum corneum lipid lamella in skin might act as a rate limiting step on the penetration of doxifluridine, fluorouracil, and tegafur. The enhancing effect of N-dodecyl-2-pyrrolidone on the permeability coefficient was more effective at higher hydrophilic drugs and the dependency of permeability coefficient on the partition coefficient disappeared in the presence of the enhancer. The effect of the enhancer on the absorption of doxifluridine, fluorouracil, and tegafur was related to the perturbation of stratum corneum lipid lamella. Carmofur was shown to be a higher lipophilic compound. The dermis appeared to act as a rate-limiting step on the skin penetration of this drug.

Walters KA, Watkinson AC, Brain KR. **In vitro skin permeation evaluation: the only realistic option.** Int J Cosmet Sci 1998;20(5):307-16.
BIOSIS COPYRIGHT: BIOL ABS. Increasing requirements for cruelty-free risk assessment in the cosmetic industry have led to the development of several alternative experimental evaluation strategies. Quantification of the potential dermal absorption of ingredients of cosmetic and other formulations by determination of human skin permeation rates in vitro is particularly relevant. Using modifications of standard in vitro protocols the human skin permeation rates of several cosmetic ingredients and potential contaminants have been determined under conditions designed to mimic consumer use. Skin penetration and permeation of octyl salicylate (a sunscreen), nonylphenol ethoxylates (surfactants) and three nitrosamines (potential contaminants) is discussed. The data demonstrate the usefulness of this technique

as a tool in the overall risk assessment of cosmetic formulations.

Zhao JF, Zhang YJ, Kubilus J, Jin XH, Santella RM, Athar M, Wang ZY, Bickers DR. **Reconstituted 3-dimensional human skin as a novel in vitro model for studies of carcinogenesis.** Biochem Biophys Res Commun 1999;254(1):49-53.

EpiDerm (MatTek Co., MA) is a reconstituted human skin equivalent which exhibits morphological and growth characteristics similar to human skin. This model has previously been utilized to evaluate the cytotoxicity and irritant potential of various cosmetic and household products. In this study, we show for the first time that EpiDerm can be used successfully to evaluate the genotoxicity of different types of known carcinogenic agents such as benzo[a]pyrene (BaP), ultraviolet B radiation (UVB), ultraviolet A radiation (UVA), and psoralen-ultraviolet A radiation (PUVA) at the molecular level. The topical application of 50 microg/cm2 BaP to EpiDerm resulted in the accumulation of BaP-DNA adducts and c-fos and p53 proteins as evidenced by immunohistochemical localization. Similarly, exposure to UVB (50 mJ/cm2) and UVA (2.5 J/cm2) enhanced the epidermal expression of c-fos and p53 proteins in the human skin equivalent. PUVA treatment of EpiDerm, however, resulted in the formation of both DNA-8-MOP adducts and augmented expression of c-fos and p53 proteins. Most of these changes reached a peak 8 h after the treatments except in the case of UVA where maximum changes in the expression of c-fos and p53 proteins were observed 24 h after treatment. These results are similar to those previously reported in human and murine skin following exposure to BaP, UVB, UVA, or PUVA indicating that human skin equivalents can be used as a convenient and cost-effective alternative to animal testing for assessing the genotoxicity and mechanism of action of mutagens/carcinogens in human skin. Copyright 1999 Academic Press.

ECOTOXICITY

Bastian KC, Alleman JE. **Microtox characterization of foundry sand residuals.** Waste Manage 1998;18(4):227-34.
BIOSIS COPYRIGHT: BIOL ABS. Although foundry residuals, consisting mostly of waste sands, represent a potentially attractive, high-volume resource for beneficial reuse applications (e.g. highway embankment construction), prospective end users are understandably concerned about unforeseen liabilities stemming from the use of these residuals. This paper, therefore, focuses on the innovative use of a microbial bioassay as a means of developing a characterization of environmental suitability extending beyond the analytical coverage already provided by mandated chemical-specific tests (i.e., TCLP, etc.). MicrotoxTM bioassays were conducted on leachates derived from residuals obtained at a wide range of facilities, including: 11 gray and ductile iron foundries plus one each steel and aluminum foundries. In addition, virgin sand samples were used to establish a relative 'natural' benchmark against which the waste foundry sands could then be compared in terms of their apparent quality. These bioassay tests were able to effectively 'fingerprint' those residuals whose bioassay behavior was comparable to that of virgin materials. In fact, the majority of gray and ductile iron foundry residuals tested during this reported study elicited MicrotoxTM response levels which fell within or below the virgin sand response range, consequently providing another quantifiable layer of support for this industry's claim that their sands are 'cleaner than dirt.' However, negative MicrotoxTM responses

beyond that of the virgin sands were observed with a number of foundry samples (i.e. four of the 11 gray or ductile iron sands plus both non-iron sands). Therefore, the latter results would suggest that these latter residuals be excluded from beneficial reuse for the immediate future, at least until the cause and nature of this negative response has been further identified.

Bat L, Raffaelli D. **Survival and growth of Corophium volutator in organically enriched sediment: A comparison of laboratory and field experiments.** Turkish J Zool 1998;22(3):219-29.
BIOSIS COPYRIGHT: BIOL ABS. In this study, the amphipod Corophium volutator (Pallas) was evaluated as test organisms for use in sediment toxicity tests by adapting standard protocols for conducting 10-day and 28-day sediment toxicity tests. Combine laboratory and field bioassays showed that Corophium can survive in organically enriched sediment if they have no alternative, suggesting that Corophium is relatively tolerant of organically enriched sediment. Neither were there effects on emergence or reburying behaviour. Therefore this bioassay is considered inappropriate for estimating the quality of organically enrichment sediment.

Beaty BJ, Black WC, Carlson JO, Clements WH, Duteau N, Harrahy E, Nuckols J, Kenneth E, Olson KE, Rayms-Keller A. **Molecular and genetic ecotoxicologic approaches to aquatic environmental bioreporting.** Environ Health Perspect 1998;106(Suppl 6):1395-407.
Molecular and population genetic ecotoxicologic approaches are being developed for the utilization of arthropods as bioreporters of heavy metal mixtures in the environment. The explosion of knowledge in molecular biology, molecular genetics, and biotechnology provides an unparalleled opportunity to use arthropods as bioreporter organisms. Interspecific differences in aquatic arthropod populations have been previously demonstrated in response to heavy metal insult in the Arkansas River (AR) California Gulch Superfund site (CGSS). Population genetic analyses were conducted on the mayfly Baetis tricaudatus. Genetic polymorphisms were detected in polymerase chain reaction amplified 16S mitochondrial rDNA (a selectively neutral gene) of B tricaudatus using single-strand conformation polymorphism analysis. Genetic differences may have resulted from impediments to gene flow in the population caused by mortality arising from exposure to heavy metal mixture pollution. In laboratory studies a candidate metal-responsive mucinlike gene, which is metal and dose specific, has been identified in Chironomus tentans and other potential AR-CGSS bioreporter species. Population genetic analyses using the mucinlike gene may provide insight into the role of this selectable gene in determining the breeding structure of B. tricaudatus in the AR-CGSS and may provide mechanistic insight into determinants of aquatic arthropod response to heavy metal insult. Metal-responsive (MR) genes and regulatory sequences are being isolated, characterized, and assayed for differential gene expression in response to heavy metal mixture pollution in the AR-CGSS. Identified promoter sequences can then be engineered into previously developed MR constructs to provide sensitive in vitro assays for environmental bioreporting of heavy metal mixtures. The results of the population genetic studies are being entered into an AR geographic information system that contains substantial biological, chemical, and geophysical information. Integrated spatial, structural, and temporal analyses of these parameters will provide invaluable information concerning environmental determinants that restrict or promote gene flow in bioreporter populations.

Boon JP, Sleiderink HM, Helle MS, Dekker M, Van Schanke A, Roex E, Hillebrand MT, Klamer HJ,

Govers B, Pastor D, et al. **The use of microsomal in vitro assay to study phase I biotransformation of chlorobornanes (Toxaphene) in marine mammals and birds. Possible consequences of biotransformation for bioaccumulation and genotoxicity.** Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1998;121(1-3):385-403.
The factors determining the bioaccumulation of lipophilic compounds in wildlife are often poorly understood, partly because it is difficult to do in vivo experiments with animals such as marine mammals and birds. To evaluate the role of phase I biotransformation in the bioaccumulation process of chlorobornanes (toxaphene), this was studied in in vitro assays with hepatic microsomes of animals that could be sampled shortly after death. The capacity of microsomes to metabolise a technical toxaphene mixture decreased in the order Phoca vitulina (harbour seal) >> Lagenorhynchus albirostris (whitebeaked dolphin) approximately equal to Diomedea immutabilis (Laysan albatross) > Physeter macrocephalus (sperm whale). Harbour seal microsomes metabolised the chlorobornane (CHB) congeners CHB-32 and CHB-62; whitebeaked dolphin and Laysan albatross microsomes only metabolised CHB-32. Metabolism of CHB-26 and CHB-50 was never observed. The negative chemical ionisation (NCI-) mass spectra of some of the hydroxylated metabolites were obtained. The number of peaks in the toxaphene residues of wildlife extracts decreased in the order of increasing in-vitro biotransformation capacity. Thus, the results of the in vitro assays and residue analysis were in accordance, although assays with microsomes of more individuals of the same species are required for a more general conclusion at the species level. Finally, the effect of in vitro biotransformation was evaluated in terms of the genotoxic potential using the Mutatox assay. Only technical toxaphene and CHB-32 were genotoxic in the direct assay, whereas the addition of rat S9 fraction or microsomes of harbour seal and albatross decreased the genotoxic response. Thus, organisms with a low ability to metabolise chlorobornanes, such as whales, may be most affected by the carcinogenic properties of toxaphene. A hypothetical reaction which fits the experimental results is discussed. Based on these results it is concluded that in vitro assays with microsomes of wildlife animals which died a natural cause can act as a valuable tool to assess the occurrence and effects of phase I metabolism. Some precautions are discussed, that should be taken to reduce the chance of false negative results.

Cikutovic MA, Fitzpatrick LC, Goven AJ, Venables BJ, Giggleman MA, Cooper EL. **Wound healing in earthworms Lumbricus terrestris: a cellular-based biomarker for assessing sublethal chemical toxicity.** Bull Environ Contam Toxicol 1999;62(4):508-14.

Clarke KR. **Nonmetric multivariate analysis in community-level ecotoxicology.** Environ Toxicol Chem 1999;18(2):118-27.
BIOSIS COPYRIGHT: BIOL ABS. Community-level data, typically in the form of abundances of over 100 species, are widely collected in the context of environmental monitoring, e.g., of the effects of disposal of drilling muds in offshore oil operations. The statistical properties of the resulting abundance arrays preclude the use of "classical" multivariate analyses, such as principal components and multivariate analysis of variance. One alternative is the use of nonparametric displays and tests, such as nonmetric multidimensional scaling (MDS) and variations of Mantel tests on similarity matrices. These do not require the restrictive assumptions of parametric techniques and possess a conceptual simplicity, facilitating their use and understanding by environmental managers and regulators. A monitoring example is discussed from Norwegian oil fields, for which such analyses have had a significant impact

on environmental practice. The techniques are equally applicable to assessing outcomes from community-level laboratory experiments and bioassays. The paper exemplifies the analysis approach taken at the Plymouth Marine Laboratory (and encapsulated in the PRIMER software) through (1) observational studies of pollution gradients in North Sea sediments and heavy metal pollutants in the Fal estuary, UK; (2) an experimental study on differential effects of metals on marine nematode communities; and (3) a bioassay approach employing a microcosm experiment on Fal estuary sediments.

Clemons E, Arkoosh MR, Casillas E. **Enhanced superoxide anion production in activated peritoneal macrophages from English sole (Pleuronectes vetulus) exposed to polycyclic aromatic compounds.** Marine Environ Res 1999;47(1):71-87.
BIOSIS COPYRIGHT: BIOL ABS. As in mammals, macrophages play a vital role in the destruction of infective organisms in fish. The current study was done to determine if exposure to polycyclic aromatic compounds (PACs), a group of chemical contaminants commonly found in the sediments of urban marine areas, alters the ability of peritoneal macrophages (Mphis) from English sole (Pleuronectes vetulus) to produce cytotoxic reactive oxygen intermediates (ROIs). Initially, assay conditions including concentration of Mphis, type of in vitro stimulant, tissue culture media, and incubation time were optimized to measure production of superoxide anion (O2-, the progenitor ROI, in English sole Mphis. English sole were then injected with an organic solvent extract of a PAC-contaminated sediment equivalent to 20 g sediment (about 860 mug selected PACs) per kg fish, via their dorsal lymphatic sinus. Peritoneal Mphis were harvested on days 1, 3, 7, and 14 post-injection. Elicited peritoneal Mphis from English sole injected with the sediment extract produced significantly more O2- after stimulation in vitro with either opsonized zymosan on days 3 and 7 after exposure, or phorbol myristate acetate on day 7 when compared to the vehicle-injected or uninjected fish. Macrophages of fish injected with the vehicle responded comparably to those from uninjected individuals. No differences in the basal amounts of O2- production from activated peritonea Mphis were observed among the treatment groups. This study demonstrates that exposure of English sole to PACs altered macrophage production of O2-. Although the direct effects of the enhanced production of this ROI are unknown, the higher levels of superoxide anion production within peritoneal macrophages may contribute to immunodysfunction and oxidative damage in P. vetulus.

Da Silva E, Soares A M, Sobral O M, Lopes I M, Correia J F, Marchante E M, Chastinet C B, Moreno A J. **Ecotoxicological responses of isolated mitochondrial systems to complex effluents. Are they worthwhile?** Chemosphere 1998;37(13):2695-701.
BIOSIS COPYRIGHT: BIOL ABS. In vitro assays with isolated rat liver mitochondria and sub-mitochondrial particles (SMP) of beef heart were compared with acute Daphnia magna test and Microtox, in order to establish different sensitivities when exposed to complex effluents. Median effective concentration (EC50) were calculated using the probit analysis, considering several endpoints: immobilisation for D. magna assays, luminescence decrease of the marine bacterium Photobacterium phosphoreum, inhibition of succinate generated potential or the depolarisation caused by ADP addition for rat liver mitochondria by the mitochondrial transmembrane electric potential (TEP) and reduction in the amount of NADH oxidised by the electron transport system to form NAD+ in the case of the forward electron transport (FET) or the NADH amount that is reduced to NAD+ for SMP, in the case of the reverse electron transfer (RET). The TEP tests showed to be more sensitive than the others one are. The

results obtained indicate the convenience of utilising in vitro systems such as the TEP or even SMP to test whole effluent ecotoxicity.

Dodard SG, Renoux AY, Hawari J, Ampleman G, Thiboutot S, Sunahara GI. **Ecotoxicity characterization of dinitrotoluenes and some of their reduced metabolites.** Chemosphere 1999;38 (9):2071-9.
In the present study, the toxic effects of 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and a selection of their respective metabolites were examined and compared to 2,4,6-trinitrotoluene (TNT) using the 15-min Microtox (Vibrio fischen) and 96-h freshwater green alga (Selenastrum capricomutum) growth inhibition tests. All of the compounds tested were less toxic than TNT. Using the Microtox assay, 2,6-DNT was more toxic than 2,4-DNT and the order of toxicity for 2,6-DNT and its metabolites was: 2,6-DNT > or = 2A-6NT >> 2,6-DAT; whereas that for 2,4-DNT was: 4A-2NT > 2A-4NT > 2,4-DNT > 2,4-DAT. For the algal test, 2,4-DNT was more toxic than 2,6-DNT and the order of toxicity for 2,4-DNT and its metabolites was: 2,4-DNT > 2,4-DAT approximately equal to 4A-2NT = 2A-4NT. The order of toxicity for 2,6-DNT and its reduced metabolites using the algal test was very similar to the Microtox bioassay. These results demonstrate that the reduced metabolites of 2,6-DNT tested in this study were less toxic than that of the parent compound, but certain partially reduced metabolites of 2,4-DNT can be more toxic than the parent molecule. These data put into question the general hypothesis that reductive metabolism of nitro-aromatics is associated with a sequential detoxification process.

Fent K, Woodin BR, Stegeman JJ. **Effects of triphenyltin and other organotins on hepatic monooxygenase system in fish.** Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1998;121(1-3):277-88.
The interaction of the organotin fungicide triphenyltin chloride (TPT) with fish microsomal monooxygenase systems has been studied in vitro and in vivo in the marine fish scup (Stenotomus chrysops). In vitro incubation of fish liver microsomes with TPT resulted in the conversion of about 40% of the native total spectral P450 to P420. In addition, a strong concentration-related inhibition of ethoxyresorufin O-deethylase (EROD) activity was observed, with a complete loss at 1.0 mM TPT. Pentoxyresorufin-O-dealkylase (PROD) activity was inhibited only at the highest concentration tested. This suggests either some specificity for the EROD catalyst CYP1A1, or a loss of reductant NADPH cytochrome c reductase as the cause. Further in vitro incubations showed that NADPH, but not NADH, cytochrome c reductase was strongly inhibited at 100 microM TPT and higher. To further investigate this effect, fish were injected with single doses of 5, 25 and 50 microM TPT (1.9, 9.6 and 19.3 mg kg-1 TPT), and 24 and 48 h later, hepatic microsomes were analyzed for total P450 content, EROD activity, NAD(P)H cytochrome c reductase, and the content of three CYP forms. EROD activity tended to be decreased in TPT-treated scup, with the response being stronger after 48 than 24 h. No significant conversion of spectrally determined P450 to cytochrome P420 was found, and cytochrome b5 was not affected. However, both NAD(P)H cytochrome c reductases were significantly inhibited at all concentrations. Immunoblot analysis showed reduction of CYP1A1 content at all doses, being significant at 25 mM after 48 h, but no decrease in CYP3A-like protein, the dominant catalyst of testosterone 6 beta-hydroxylation, nor CYP2B-like protein, the major contributor to indicates significant effects of TPT at high concentrations on fish hepatic CYP1A1 protein, EROD activity and the reductases. TPT seems to act more specifically on CYP1A1 than on other CYP forms. These findings

combined with those of our previous studies (Bruschweiler BJ, Wurgler FE, Fent K. Environ Toxicol Chem 1996;15:827-735; Fent K, Bucheli TD. Aquat Toxicol 1994;28:107-126; Fent K, Stegeman JJ. Aquat Toxicol 1991;20:159-168; Fent K, Stegeman JJ. Aquat Toxicol 1993;24:219-240) indicate a general degenerative effect of organotins on the fish microsomal monooxygenase system, although some differences are seen between the organotins, and between species. We conclude that these effects of organotins have consequences for use of CYP1A as a biomarker and endocrine disruption.

Fernandez-Vega C, Sancho E, Ferrando MD, Andreu-Moliner E. **Thiobencarb toxicity and plasma AChE inhibition in the European eel.** J Environ Sci Health B 1999;34(1):61-73.
The acute toxicity of the herbicide thiobencarb (S-4-chlorobenzyl diethylthiocarbamate) was determined for the European eel (Anguilla anguilla). The 24, 48, 72 and 96 hours median lethal concentrations (LC50) were 25.7, 21.7, 17.0 and 13.2 mg/L, respectively. Fish were also exposed to a sublethal thiobencarb concentration (1/60 LC50-96 hr = 0.22 mg/L) during 96 hours in a flow-through system and then an elimination period of 192 hours in clean water was allowed. Eels were removed and blood samples taken out at each exposure time and recovery period in order to evaluate AChE activity. Thiobencarb induced significant inhibitory effects on plasma AChE activity of A. anguilla from the first contact time with the toxicant. This inhibition (under 50% activity) was maintained during the entire exposure period (96 hours) and even those animals transferred to clean water showed plasma AChE activity different from the controls. Differences between total and specific AChE activity were detected during the exposure period. Total AChE activity in the plasma from animals transferred to a medium free of toxicant recovered its normal value while specific AChE activity remained depressed (< 50%) until five days later.

Fisher DJ, Burton DT, Yonkos LT, Turley SD, Ziegler GP. **The relative acute toxicity of continuous and intermittent exposures of chlorine and bromine to aquatic organisms in the presence and absence of ammonia.** Water Res 1999;33(3):760-8.
BIOSIS COPYRIGHT: BIOL ABS. Sodium bromide can be used to convert hypochlorous acid into hypobromous acid. An alternative strategy to biofouling control uses the simultaneous addition of sodium bromide and chlorine to water used for power plant condenser cooling. This approach can significantly reduce the total disinfectant and halogen application rates because the bromine oxidants that are generated are more effective for controlling biofouling than their chlorine counterparts, especially at higher pHs. Since such a change in biofouling control strategy could adversely impact the environment, the acute toxicity of bromine oxidants were evaluated in both continuous and intermittent exposure scenarios. Decay properties of bromine oxidants were compared to those of chlorine oxidants. In addition, two tests were conducted to investigate the relative toxicities of chloramines and bromamines. For the six species tested, bromine oxidants were two to five times more toxic than chlorine oxidants. For continuous exposure to bromine oxidants, LC50 values for daphnids (Dapnia magna) and amphipods (Hyalella azteca) could not be calculated because significant mortality occurred at the oxidant quantitation limit. Both chlorine and bromine proved to be more toxic to daphnids and mysids (Mysidopsis bahia) in the presence of ammonia. While the toxicity data show that bromine oxidants are more toxic than chlorine oxidants, bromine oxidants decayed two to five times faster than chlorine oxidants in freshwater and saltwater, respectively. With regard to potential environmental impact. it is important that one consider the more efficacious biocidal characteristics of bromine (i.e.

higher pHs) in the context of more rapid decay relative to chlorine. The strategy of using simultaneous addition of sodium bromide and chlorine could reduce environmental impact potential, although insufficient data exists to prove this.

Gauthier JM, Dubeau H, Rassart E. **Induction of micronuclei in vitro by organochlorine compounds in beluga whale skin fibroblasts.** Mutat Res 1999;439(1):87-95.
Beluga whales (Delphinapterus leucas) inhabiting the St. Lawrence estuary are highly contaminated with environmental pollutants and have a high incidence of cancer. Environmental contaminants may be partly responsible for the high cancer incidence observed in this population. DNA damage plays an important role in the development of cancer. The micronuclei (MN) assay was used to test the genotoxic potential of organochlorine (OC) pesticides with and without external metabolic factor in skin fibroblasts of an arctic beluga whale. Toxaphene, chlordane and p,p'-DDT induced significant ($p<0.05$) concentration-response increases of micronucleated cells (MNCs). Statistically significant increases in MNCs, ranging from 1.7- to 5-folds when compared to control cultures, were observed for 0.05, 0.5, 5 and 10 microg/ml toxaphene, 2, 5 and 10 microg/ml chlordane and 10 and 15 microg/ml p,p'-DDT. Presence of exogeneous metabolic factor (S9) completely abolished the MN induction potency of chlordane and p,p'-DDT, and toxaphene induced MN formation at higher concentrations (0.5 microg/ml) than without S9 mix. The ecotoxicological significance of MN induction by low concentrations of toxaphene is unknown and do not imply that toxaphene is involved in the etiology of cancer in St. Lawrence beluga whales. However, because of the known genotoxicity of toxaphene and the long lifespan of beluga whales, it cannot be excluded that toxaphene may pose a long-term genetic hazard to the more contaminated whales of this population.

George-Ares A, Clark JR, Biddinger GR, Hinman ML. **Comparison of test methods and early toxicity characterization for five dispersants.** Ecotoxicol Environ Saf 1999;42(2):138-42.
The acute toxicities of a commercial dispersant (Corexit 9527) and four experimental dispersant formulations were evaluated using the 96-h mysid (Mysidopsis bahia) test and two rapid screening tests, Microtox and the Mysid IQ Toxicity Test. During 96-h toxicity tests, survival observations were recorded at 3, 6, 9, 12, and 24 h to document mortalities from short-term exposures more consistent with field exposure times and more approximate to exposure times used in Microtox and the Mysid IQ Toxicity Test. At nominal concentrations (6.25 and 12.5 mg/liter) and exposure times (3-24 h) near the upper range of predicted field conditions, mysid mortalities were </=5% for all test materials. Microtox and Mysid IQ Toxicity Test were evaluated for their ability to differentiate test materials compared with that of the 96-h mysid test. Dispersant formulations were ranked by relative toxicities based on LC50 or EC50 values and ranks compared among test methods. Microtox ranked the test materials similar to the 96-h mysid test. Ranks from the Mysid IQ Toxicity Test were dissimilar to those of the other tests. Early mortality observations during 96-h tests did not provide a better basis for comparing results of the rapid screening tests.

Guilhermino L, Sobral O, Chastinet C, Ribeiro R, Goncalves F, Silva MC, Soares AM. **A Daphnia magna first-brood chronic test: an alternative to the conventional 21-Day chronic bioassay?** Ecotoxicol Environ Saf 1999;42(1):67-74.
In this study, a comparison was made of the results obtained in Daphnia magna chronic bioassays after

first-brood release and after 21 days of exposure, using inhibition of normal reproduction and growth as effect criteria and EC10, EC20, EC50, no-observed-effect concentration (NOEC), and lowest-observed-effect concentration (LOEC) as statistical parameters. Test substances were sodium bromide (NaBr), 3,4-dichloroaniline (DCA), cadmium, and parathion. For NaBr, DCA, and cadmium, toxicity evaluated after the first-brood release was similar to toxicity evaluated after 21 days, using reproduction as end point. Parathion did not affect either reproduction or growth. Thus, LC50, NOEC, and LOEC were calculated using mortality as the endpoint for parathion. Results indicate that the period until release of the first brood is sufficient to predict the toxicity of some chemicals to D. magna. Values estimated on the basis of a logistic model (EC10, EC20, and EC50) were more appropriate than NOECs and LOECs for evaluating toxicity of the test substances. Furthermore, classic endpoints used for the evaluation of chronic toxicity (inhibition of normal reproduction and growth) may not be adequate to evaluate the sublethal toxicity of compounds that induce cumulative effects leading to mortality within the test period and causing no observable effects on the reproduction and growth of the species. Endpoints indicative of biochemical stress or effects on specific targets of the test compound may be useful in sublethal toxicity evaluation. Copyright 1999 Academic Press.

Guzzella L. **Comparison of test procedures for sediment toxicity evaluation with Vibrio fischeri bacteria.** Chemosphere 1998;37(14-15):2895-909.
BIOSIS COPYRIGHT: BIOL ABS. The use of bacterial luminescence assays is particularly effective in the contaminated sediment evaluation. In the present study, a comparison was made of different bacterial luminescence assays including acute toxicity of elutriates with Microtox and LUMIStox; chronic toxicity of elutriates with LUMIStox; and, acute toxicity of solvent extracts with both the tests and the Solid Phase test with Microtox. The toxicity assay procedures were utilized for the Po River sediment toxicity evaluation, testing samples with different physical and chemical characteristics. The results evidentiated that each sediment test procedures provided independent and complementary ecotoxicological responses useful for a sediment classification of the most polluted samples.

Ieradi LA, Moreno S, Bolivar JP, Cappai A, Di Benedetto A, Cristaldi M. **Free-living rodents as bioindicators of genetic risk in natural protected areas.** Environ Pollut 1998;102(2-3):265-8.
BIOSIS COPYRIGHT: BIOL ABS. A study was carried out in the south of the Iberian Peninsula in an industrial area (Huelva city) and in two natural areas ('Reserva Biologica de Donana' in Donana National Park and 'Isla Cristina' Marshland Natural Park), located windward and leeward of the industrial area, to estimate genetic risk induced by environmental pollution in wild mice. Mutagenetic effects in Algerian mice (Mus spretus) free living in the industrial area and the range of contamination on other populations of the same species living in the two natural protected areas, were investigated. Micronucleus test on bone marrow and peripheral blood was used to detect genetic alterations. A statistically significant increase in the frequency of micronuclei was observed in animals from the industrial area and from the Donana Biological Reserve in comparison with those from 'Isla Cristina' marshlands. The results suggest that the mutagenicity level in natural populations living in protected areas should be controlled, and wild mice could be used as key organisms in pollution monitoring and environmental conservation.

Li J, Quabius ES, Wendlaa Bonga S, Flik G, Lock R. **Effects of water-borne copper on branchial chloride cells and Na+/K+-ATPase activities on Mozambique tilapia (Oreochromis mossambicus).**

Aquatic Toxicol 1998;43(1):1-11.
BIOSIS COPYRIGHT: BIOL ABS. Freshwater tilapia (Oreochromis mossambicus) were exposed for different periods up to 28 days to 3.2 muM of water-borne Cu. Electron microscopical analysis of the gills demonstrated significant changes in the structure and number of chloride cells (CCs) from Cu-exposed fish when compared to controls. These cells, which are the main location of the Na+/K+-ATPase of the gills and which play a crucial role in transepithelial Na+ transport, showed a time-related increase of degeneration by apoptosis and necrosis in the Cu-exposed fish. After 28 days of Cu exposure, apoptotic CCs had doubled in number while necrotic CCs had even increased by a factor of ten. The activity of the gill Na+/K+-ATPase and the plasma Na+ concentration decreased in time and in parallel. An inverse relationship between the Na+/K+-ATPase specific activity and the branchial Cu content further supports the notion that this enzyme is very sensitive to Cu2+ inhibition. In contrast to controls, no significant correlation was found in the Cu-exposed fish between the opercular CC number and the gill Na+/K+-ATPase total activities, despite the large increase in number of these cells. This study provides further evidence that not only the number but also the quality of the CCs, may determine to a large extent the branchial capacity of a freshwater fish to absorb Na+ from the surrounding water.

Ma M, Tong Z, Wang Z, Zhu W. **Acute toxicity bioassay using the freshwater luminescent bacterium Vibrio-qinghaiensis sp. Nov.-Q67.** Bull Environ Contam Toxicol 1999;62(3):247-53.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE VIBRIO QINGHAIENSIS S CYMNOCYPRIS PRZEWALSKII VIBRIO FISCHERI STRAIN-Q67 TOXICITY SCREENING TOOL STRAIN-T3 POLLUTION TOXICOLOGY ENVIRONMENTAL CONTAMINATION MICROTOX BIOASSAY FRESHWATER ECOLOGY CHINESE EPA MERCURY HEAVY METAL POLLUTANT COPPER ZINC CADMIUM NICKEL MANGANESE FOSETHYL ALUMINUM PESTICIDE QUINTOZENE TRIADIMEFON CCU 1-2-CHLOROBENZOYL-3-4-CHLOROPHENYLUREA ALDICARB DMA N-2 4-DIMETHYLPHENYL-N'-METHYLFORMAMIDINE 3-CHLOROPHENOL 2 3-DICHLOROPHENOL 2 5-DICHLOROPHENOL 2 4 5-TRICHLOROPHENOL 2 3 4 5-TETRACHLOROPHENOL ACUTE TOXICITY BIOASSAY BIOASSAY METHOD GOVERNMENT AGENCY.

Marty J, Djomo JE, Bekaert C, Pfohl-Leszkowicz A. **Relationships between formation of micronuclei and DNA adducts and EROD activity in newts following exposure to benzo(a)pyrene.** Environ Mol Mutagen 1998;32(4):397-405.
The aim of this study was to determine whether the micronucleus test, using the larvae of a lower invertebrate, the newt Pleurodeles waltl, is suitable for evaluating the overall genotoxicity of polluted water (AFNOR Standard, 1992). The study used the pollutant model benzo(a)pyrene (BaP). After having shown that BaP is metabolized by the larvae, the test was carried out under standard AFNOR conditions. We investigated the relationship between the BaP concentration, spectrofluorometric measurement of liver EROD activity, and two genotoxicity biomarkers: DNA adduct production (32P-postlabeling detection) and micronucleus formation in red blood cells (RBCs) (number of micronucleated RBCs per 1,000). A dose effect was found for all three biomarkers, which were seen to be linearly correlated showing that the biochemical mechanisms occurring in the newt larvae exposed to BaP are similar to those described in higher vertebrates. This result confirms the utility of the test for the evaluation of the overall hazard of a given aquatic environment.

Newman MC, Mccloskey JT, Tatara CP. **Using metal-ligand binding characteristics to predict metal toxicity: quantitative ion character-activity relationships (QICARs).** Environ Health Perspect 1998;106(Suppl 6):1419-25.
Ecological risk assessment can be enhanced with predictive models for metal toxicity. Modelings of published data were done under the simplifying assumption that intermetal trends in toxicity reflect relative metal-ligand complex stabilities. This idea has been invoked successfully since 1904 but has yet to be applied widely in quantitative ecotoxicology. Intermetal trends in toxicity were successfully modeled with ion characteristics reflecting metal binding to ligands for a wide range of effects. Most models were useful for predictive purposes based on an F-ratio criterion and cross-validation, but anomalous predictions did occur if speciation was ignored. In general, models for metals with the same valence (i.e., divalent metals) were better than those combining mono-, di-, and trivalent metals. The softness parameter (sigma p) and the absolute value of the log of the first hydrolysis constant ([symbol: see text] log KOH [symbol: see text]) were especially useful in model construction. Also, delta E0 contributed substantially to several of the two-variable models. In contrast, quantitative attempts to predict metal interactions in binary mixtures based on metal-ligand complex stabilities were not successful.

Pardos M, Benninghoff C, Thomas RL, Khim-Heang S. **Confirmation of elemental sulfur toxicity in the microtox assay during organic extracts assessment of freshwater sediments.** Environ Toxicol Chem 1999;18(2):188-93.
BIOSIS COPYRIGHT: BIOL ABS. Recent literature indicates that the elemental sulfur occurring in organic extracts of sediment samples can be toxic to the bacterium Vibrio fisheri, used in standard Microtox bioassays. This observation was tested by means of the solvent extraction of 14 freshwater sediment samples from rivers tributary to Lake Geneva (Switzerland-France), measuring both Microtox toxicity and the elemental sulfur concentration of the extracts. Aliquots of these sediment extracts were further treated to remove the sulfur by adding acid-activated copper to the crude extracts; for 18 h in one case, and for 116 h in an other. The results were a significant amount of the observed acute toxicity in the Microtox assay of 81% of sample extracts (n = 42, crude and after cleanup) was due to elemental sulfur, and despite a median decrease of 99.1% of elemental sulfur in the extracts subject to a 116-h cleanup, sulfur toxicity was not completely excluded for 57% (8/14) of the samples. Clearly, the Microtox methodology needs to be amended to more accurately assess the potential impact of organic pollutants in sediments when solvent extracts are used. This will help to cut down on costly and unnecessary remedial actions.

Poher I, Blanc G. **Pharmacokinetics of a discontinuous absorption process of oxolinic acid in turbot, Scophthalmus maximus, after a single oral administration.** Xenobiotica 1998;28(11):1061-73.
BIOSIS COPYRIGHT: BIOL ABS. 1. The pharmacokinetics of oxolinic acid have been studied in 500 g turbot (Scophthalmus maximus). The fish were kept in seawater at 16~C with a 15 h/9 h photoperiod. Oxolinic acid was administered orally via a stomach tube at a single dose of 10 mg/kg of body weight. Serum concentrations of oxolinic acid were determined by a (HPLC) using liquid phase extraction with an internal standard and a fluorescence detection. 2. The pharmacokinetic process was not significantly

sex-influenced. The short elimination phase of the oxolinic acid in turbot after oral administration was similar to the elimination after intravascular administration. The serum concentration profile of oxolinic acid was better described by a discontinuous absorption model than by compartment models using continuous absorption processes. The absorption of oxolinic acid in turbot was characterized by two distinct phases after a lag time of about 2 h. A time (Tmax) of 12 h was necessary to reach the peak serum concentration (Cmax) of 1.41 mug/ml. The oral bioavailability was 27.9%. 3. Based on the minimum inhibitory concentration for susceptible strains, and especially Vibrio anguillarum, the oxolinic acid could be effective in turbot after an oral treatment of 10 mg/kg/day.

Reader S, Moutardier V, Denizeau F. **Tributyltin triggers apoptosis in trout hepatocytes: the role of Ca2+, protein kinase C and proteases.** Biochim Biophys Acta 1999;1448(3):473-85.
The purpose of the present study was to study the mechanisms involved in the induction of apoptosis and by tributyltin (TBT) in rainbow trout hepatocytes, and to examine the role of intracellular Ca2+, protein kinase C (PKC) and proteases in the apoptotic process. The intracellular Ca2+ chelator BAPTA-AM has a suppressive effect on TBT-mediated apoptosis. However, exposure to the ionophore A23187 is not sufficient to induce apoptosis in trout hepatocytes. The results obtained also show that TBT stimulates PKC gamma and delta translocation from cytosol to the plasma membrane in trout hepatocytes after 30 min of exposure. However, PKC gamma translocation is down-regulated after 90 min of treatment. The addition of protein kinase inhibitors (staurosporine and H-7) not only fails to inhibit apoptosis induced by TBT, but also leads to enhancement of DNA fragmentation. These inhibitors also afford a remarkable protection against the loss of plasma membrane integrity caused by TBT exposure. PMA, a direct activator of PKC, fails to stimulate DNA fragmentation. In addition, Z-VAD.FMK is an extremely potent inhibitor of TBT-induced apoptosis in trout hepatocytes, indicating that the activation of ICE-like proteases is a key event in this process. The cysteine protease inhibitor N-ethylmaleimide also prevented TBT-induced DNA fragmentation. Taken together, these data allow for the first time to suggest a mechanistic model of TBT-induced apoptosis. We propose that TBT could trigger apoptosis through a step involving Ca2+ efflux from the endoplasmic reticulum or other intracellular pools and by mechanisms involving cysteine proteases, such as calpains, as well as the phosphorylation status of apoptotic proteins such as Bcl-2 homologues.

Robidoux PY, Lopez-Gastey J, Choucri A, Sunahara GI. **Screening of illicit toxic substances discharged in chemical toilet sludge.** Qual Assur 1998;6(1):23-44.
This article presents an integrative approach, using toxicological and chemical analyses, to screen for toxic substances that could be illegally added to the chemical-toilet sludge received at the wastewater treatment plant of the Montreal urban community. Four toxicity tests (Microtox, bacterial-respiration, root-elongation, and seed-germination tests) were used to establish the toxicity range of a "normal" sludge and the determination of threshold limits criteria. Chemical-toilet sludge samples were spiked with two types and amounts of contaminants (zinc, phenol). Conservative criteria were used to detect abnormal toxicity with great reliability and avoid false positives (i.e., detecting abnormal toxicity in nonspiked sludge). Taken individually, the seed-germination test was the least discriminating toxicological method (detecting only 10% of the spiked samples); the bacterial-respiration test was relatively better (detecting 72% of the spiked samples). Using a limited battery of two toxicity tests (Microtox and respiration test), the identification of contaminated chemical-toilet sludge can be detected

with good efficiency and possibly great reliability (more than 80% of the spiked samples). This proposed procedure is efficient, easy to apply, cost-effective, and very fast (an abnormal toxicity level can be determined within a few hours).

Segner H. **Fish cell lines as a tool in aquatic toxicology.** Exs 1998;86:1-38.
In aquatic toxicology, cytotoxicity tests using continuous fish cell lines have been suggested as a tool for (1) screening or toxicity ranking of anthropogenic chemicals, compound mixtures and environmental samples, (2) establishment of structure-activity relationships, and (3) replacement or supplementation of in vivo animal tests. Due to the small sample volumes necessary for cytotoxicity tests, they appear to be particularly suited for use in chemical fractionation studies. The present contribution reviews the existing literature on cytotoxicity studies with fish cells and considers the influence of cell line and cytotoxicity endpoint selection on the test results. Furthermore, in vitro/in vivo correlations between fish cell lines and intact fish are discussed. During recent years, fish cell lines have been increasingly used for purposes beyond their meanwhile established role for cytotoxicity measurements. They have been successfully introduced for detection of genotoxic effects, and cell lines are now applied for investigations on toxic mechanisms and on biomarkers such as cytochrome P4501A. The development of recombinant fish cell lines may further support their role as a bioanalytical tool in environmental diagnostics.

Stabell OB, Aanesen RT, Eilertsen HC. **Toxic peculiarities of the marine alga Phaeocystis pouchetii detected by in vivo and in vitro bioassay methods.** Aquatic Toxicol 1999;44(4):279-88.
BIOSIS COPYRIGHT: BIOL ABS. The marine alga Phaeocystis pouchetii has recently been shown to display toxic properties towards fish (i.e. cod, Gadus morhua) larvae. The assumed toxic principle of this prymnesiophyte was extracted from Phaeocystis cultures by filtering and solid phase sorbent techniques. Toxicity testing was carried out by in vitro and in vivo bioassay methods based on blood haemolysis and injection into flies. The active material from the Phaeocystis cultures was found within a chemical fraction previously established for the separation of Chrysochromulina sp. and Prymnesium sp. toxins. The presence of active material was also found in filtered seawater collected during a Phaeocystis bloom, confirming that Phaeocystis releases the active material into the natural environment. Haemolytic activity was almost absent in the material tested, demonstrating that the toxic principle in Phaeocystis is different from that described for other prymnesiophytes. By the fly bioassay method, on the other hand, a rapid response to injected material was obtained, resulting in a high proportion of apparently 'dead' flies registered within 1 h. The time scale of response in flies coincided with that previously reported for haemolytic toxins of Prymnesium parvum. However, an unexpected response was observed with the Phaeocystis material, since some of the flies that were assumed dead regained motility within a 4-h period of monitoring, the portion of awakened flies being inversely related to dose injected. Regained motility was also found with injected material from filtered natural seawater. Accordingly, the proposed toxins released by Phaeocystis appear to be compounds that hold anaesthetic properties, possibly expressing toxic effects when presented in surplus doses. These findings suggest that Phaeocystis may primarily be harmful to fish larvae following ingestion.

Twagilimana L, Bohatier J, Groliere CA, Bonnemoy F, Sargos D. **A new low-cost microbiotest with the Protozoan spirostomum teres: culture conditions and assessment of sensitivity of the ciliate to**

14 pure chemicals. Ecotoxicol Environ Saf 1998;41(3):231-44.
This paper defines the culture conditions of the ciliate Spirostomum teres and assesses its sensitivity to some xenobiotics for the development of a new low-cost microbiotest. The model was selected for its ubiquitous distribution, large size for a unicellular species, easy culture in holoxenic medium, moderate generation time, and high sensitivity to pure toxicants. The influence of different culture waters, inocula of ciliates, food, temperature, light, and darkness on the growth of the ciliate population was tested. The shortest generation time (average 39 h) was obtained for cultures incubated at 25 degreesC in the dark with an inoculum of 4 ciliates per ml in 25 ml of Volvic mineral water containing 8 boiled wheat grains, when preincubated without ciliates for the previous week. Under these conditions, it was possible to obtain about 3000 ciliates/ml 3 weeks later. Acute toxicity tests (24-h LC50) were carried out for CuSO4, HgCl2, CdCl2, K2Cr2O7, ZnSO4, Pb(NO3)2, thiram, carbaryl, lindane, parathion, parathion methyl, paraoxon, 2, 4,6-trichlorophenol, and sodium pentachlorophenolate (Na-PCP). Very high sensitivity of the model to Hg2+, Cu2+, Cd2+, thiram, and Na-PCP was established. Comparison of its sensitivity with that of Microtox (current results), Daphnia Magna, Tetrahymena pyriformis, Colpidium campylum, and murine fibroblasts (data from literature) confirms the high sensitivity of the model, especially to heavy metals. Easy-to-perform, cost-effective, and sensitive bioassays using S. teres are suitable for risk assessment and early detection of toxicity in fresh water. Copyright 1998 Academic Press.

Wei L, Yu H, Cao J, Sun Y, Fen J, Wang L. **Determination and prediction of partition coefficient and toxicity for sulfonylurea herbicides and their degradation products.** Chemosphere 1999;38 (7):1713-9.
BIOSIS COPYRIGHT: BIOL ABS. The n-octanol/water partitioning coefficients (logKow) of three sulfonylurea herbicides, metsulfuron methyl chlorsulfuron and bensulfuron methyl and five of the degradation products were determined by the shake-flask method. The capacity factors (logK') were determined by RP-HPLC with ODS column and methanol-water eluent. Correlations between logKow and logK' and molecular connectivity index were studied. Acute toxicity to the bacterium Photobacterium phosphoreum (15-min-logEC50) and the green alga Chlorella pyrenoidosa (96-h-logEC50) were determined and correlated with logKow and logK'. The quantitative structure-activity-relationship equations showed that logKow and logK' are linearly correlated with toxicity, and both logKow and logK' can be used in the prediction. No significant acute toxicity of the tested compounds to the cladocera Daphnia magna was observed in the laboratory tests, and this was explained by ionic forms of the compounds in neutral aqueous solution.

Wink M, Schmeller T, Latz-Bruening B. **Modes of action of allelochemical alkaloids: interaction with neuroreceptors, DNA, and other molecular targets.** J Chem Ecol 1998;24(11):1881-1937.
BIOSIS COPYRIGHT: BIOL ABS. Several alkaloids are toxic to insects and vertebrates and, in addition, can inhibit the growth of bacteria and plant seedlings. In vitro assays were established to elucidate their modes of action and to understand their allelochemical properties. Basic molecular targets studied, present in all cells, included DNA intercalation, protein biosynthesis, and membrane stability. The degree of DNA intercalation was positively correlated with inhibition of DNA polymerase I, reverse transcriptase, and translation at the molecular level and with toxicity against insects and vertebrates at an organismic level. Inhibition of protein biosynthesis was positively correlated with animal toxicity.

Molecular targets studied, present only in animals, included neuroreceptors (alpha1, alpha2, serotonin, muscarinic, and nicotinic acetylcholine receptors) and enzymes related to acetylcholine (acetylcholine esterase and choline acetyltransferase). The degree of binding of alkaloids to adrenergic, serotonin, and muscarinic acetylcholine receptors was positively correlated in G-protein-coupled receptors. Receptor binding and toxicity was correlated in insects. The biochemical properties of alkaloids are discussed. It is postulated that their structures were shaped in a process termed "evolutionary molecular modeling" to interact with a single and, more often, with several molecular targets at the same time. Many alkaloids are compounds with a broad activity spectrum that apparently have evolved as "multipurpose" defense compounds. The evolution of allelochemicals affecting more than one target could be a strategy to counteract adaptations by specialists and to help fight off different groups of enemies.

Yan Cheng-Nong, Liu YI, Wang Tian-Zhi, Tan Zhi-Qun, Qu Song-Sheng, Shen Ping. **Thermochemical studies of the toxic actions of heavy metal ions on Rhizopus nigricans.** Chemosphere 1999;38(4):891-8.
BIOSIS COPYRIGHT: BIOL ABS. By using a LKB2277 BioActivity Monitor (heat conduction microcalorimeter), stopped-flow method, the thermogenetic curves of Rhizopus nigricans growth at 25~ C inhibited by four kinds of heavy metal ions are determined, parameters such as growth rate constants k, inhibitory ratio I, half inhibitory concentration IC50 et al. are obtained. The experimental results show that heavy metal ions can inhibit Rhizopus nigricans growth obviously, low concentration of Cu2+ has promoting action. The inhibitory sequence is Cd2+ > Hg2+ > Pb2+ > Cu2+, half inhibitory concentration of them are Cd2+ 0.8 mu g.ml-1, Hg2+ 1.7 mu g.ml-1, Pb2+ 48.0 mu g.ml-1, Cu2+ 110 mu g.ml-1. This microclorimetric bioassay for acute cellular toxicity is based on metabolic heat evolution from cultured cells. The assay is quantitative, inexpensive, and versatile; moreover, toxicological information can be obtained with cell from other species of interest.

Yike I, Allan T, Sorenson WG, Dearborn DG. **Highly sensitive protein translation assay for trichothecene toxicity in airborne particulates: comparison with cytotoxicity assays.** Appl Environ Microbiol 1999;65(1):88-94.
BIOSIS COPYRIGHT: BIOL ABS. Screening assays for environmental mycotoxins in bulk samples currently use cytotoxicity in cell cultures, but their application to air particulate samples often lacks sensitivity and specificity for fungal spores. An assay based on inhibition of protein synthesis using translation of firefly luciferase in a rabbit reticulocyte system has been developed for the detection of trichothecene mycotoxins found in the spores of toxigenic fungi. Ethanol extracts of air particulates trapped on polycarbonate filters are ultrafiltered and applied at several dilutions to a translation reaction mixture. The activity of translated luciferase is measured directly in a luminometer, eliminating the need for radioisotopes and time-consuming sample processing. Parallel standard curves using a commercially available trichothecene provide for expression of the results in T-2 toxin equivalents per cubic meter of air. The assay can be completed in 2 h and is readily applicable to multiple samples. Comparison to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cytotoxicity assay indicates a 400-fold increase in sensitivity of trichothecene detection in addition to a much higher specificity for these toxins. Initial field testing indicates a strong correlation between the measured level of toxicity and the presence of toxigenic fungi detected with microbiological methods. In conclusion, this luciferase translation assay offers a rapid and highly sensitive and specific method for quantitative detection of trichothecene

mycotoxin activity in air particulate samples.

GENOTOXICITY AND MUTAGENESIS

Abramsson-Zetterberg L, Grawe J, Zetterberg G. **The micronucleus test in rat erythrocytes from bone marrow, spleen and peripheral blood: the response to low doses of ionizing radiation, cyclophosphamide and vincristine determined by flow cytometry.** Mutat Res 1999;423(1-2):113-24.
The frequency of micronucleated polychromatic erythrocytes (fMPCE) was determined in samples from bone marrow, spleen and peripheral blood of rats exposed to low doses of X-rays, cyclophosphamide or vincristine. The fMPCE values were lower in the peripheral blood than in bone marrow or spleen. This is due to the elimination of MPCE from the circulating blood, which was confirmed by the results from prolonged exposure of rats to gamma-radiation. When the analysis was restricted to the youngest PCE in peripheral blood, the sensitivity of the assay was considerably improved. This can be reproducibly achieved with the flow cytometric analysis. Copyright 1999 Elsevier Science B.V.

Anderson D, Yu TW, McGregor DB. **Comet assay responses as indicators of carcinogen exposure.** Mutagenesis 1998;13(6):539-55.
Over 200 agents/factors have been examined in the single cell gel electrophoresis assay, more commonly known as the Comet assay, performed either in vitro or in vivo in a variety of species. Unequivocal carcinogenicity data are available for 119 of them, amongst which unequivocal Comet assay data exist for 95 agents. Of these 95 agents the prevalence of carcinogens was 88% (84/95). The carcinogens that were Comet positive (sensitivity) formed 88% (74/84), the non-carcinogens that were Comet negative (specificity) formed 64% (7/11). This simple analysis of the Comet assay has not taken account of the difference between in vitro and in vivo responses, species differences or organ and tissue differences. Also, limitations as to the conduct of the assay have not been examined in any depth. Thus, at the present time the Comet assay has high sensitivity for carcinogens, but its specificity is uncertain because few non-carcinogens have been tested.

Banath JP, Wallace SS, Thompson J, Olive PL. **Radiation-induced DNA base damage detected in individual aerobic and hypoxic cells with endonuclease III and formamidopyrimidine-glycosylase.** Radiat Res 1999;151(5):550-8.
X-ray-induced DNA base damage can be detected using endonuclease III and formamidopyrimidine-glycosylase, which create DNA strand breaks at enzyme-sensitive sites. Strand breaks can then be measured with excellent sensitivity using the alkaline comet assay, a single-cell gel electrophoresis method that detects DNA damage in individual cells. In using this approach to measure the oxygen enhancement ratio (OER) for radiation-induced base damage, we observed that the number of enzyme-sensitive sites increased with dose up to 4 Gy in air and 12 Gy in hypoxic WIL2NS cells. After rejoining of radiation-induced strand breaks, base damage was detected more easily after higher doses. The number of radiation-induced enzyme-sensitive sites was similar under both air and nitrogen. Base damage produced by hydrogen peroxide and 4-nitroquinoline-N-oxide (4NQO) was also measured. Results with hydrogen peroxide (20 min at 4 degrees C) were similar to those observed for X rays, indicating that enzyme-sensitive sites could be detected most efficiently when few direct strand breaks were present. Removing DNA-associated proteins before irradiation did not affect the ability to detect

base damage. Base damage produced by 4NQO (30 min at 37 degrees C) was readily apparent after treatment with low concentrations of the drug when few 4NQO-induced strand breaks were present, but the detection sensitivity decreased rapidly as direct strand breaks increased after treatment with higher concentrations. We conclude that: (1) the OER for base damage is approximately 1.0, and (2) the presence of direct DNA strand breaks (>2000-4000 per cell) prevents accurate detection of base damage measured as enzyme-sensitive sites with the alkaline comet method.

Boulton S, Kyle S, Durkacz BW. **Interactive effects of inhibitors of poly(ADP-ribose) polymerase and DNA-dependent protein kinase on cellular responses to DNA damage.** Carcinogenesis 1999;20 (2):199-203.
DNA-dependent protein kinase (DNA-PK) and poly(ADP-ribose) polymerase (PARP) are activated by DNA strand breaks and participate in DNA repair. We investigated the interactive effects of inhibitors of these enzymes [wortmannin (WM), which inhibits DNA-PK, and 8-hydroxy-2-methylquinazolin-4-one (NU1025), a PARP inhibitor] on cell survival and DNA double-strand break (DSB) and single-strand break (SSB) rejoining in Chinese hamster ovary-K1 cells following exposure to ionizing radiation (IR) or temozolomide. WM (20 microM) or NU1025 (300 microM) potentiated the cytotoxicity of IR with dose enhancement factors at 10% survival (DEF10) values of 4.5 +/- 0.6 and 1.7 +/- 0.2, respectively. When used in combination, a DEF10 of 7.8 +/- 1.5 was obtained. WM or NU1025 potentiated the cytotoxicity of temozolomide, and an additive effect on the DEF10 value was obtained with the combined inhibitors. Using the same inhibitor concentrations, their single and combined effects on DSB and SSB levels following IR were assessed by neutral and alkaline elution. Cells exposed to IR were post-incubated for 30 min to allow repair to occur. WM or NU1025 increased net DSB levels relative to IR alone (DSB levels of 1.29 +/- 0.04 and 1.20 +/- 0.05, respectively, compared with 1.01 +/- 0.03 for IR alone) and the combination had an additive effect. WM had no effect on SSB levels, either alone or in combination with NU1025. SSB levels were increased to 1.27 +/- 0.05 with NU1025 compared with IR alone, 1.02 +/- 0.04. The dose-dependent effects of the inhibitors on DSB levels showed that they were near maximal by 20 microM WM and 300 microM NU1025. DSB repair kinetics were studied. Both inhibitors increased net DSB levels over a 3 h time period; when they were combined, net DSB levels at 3 h were identical to DSB levels immediately post-IR. The combined use of DNA repair inhibitors may have therapeutic potential.

Brena-Valle M, Serment-Guerrero J. **SOS induction by gamma-radiation in Escherichia coli strains defective in repair and/or recombination mechanisms.** Mutagenesis 1998;13(6):637-41.
Ionizing radiation causes several types of DNA lesions, mainly single- or double-strand breaks and base damage. By means of the chromotest, an assay that allows the level of the SOS response to be monitored via beta-galactosidase enzymatic activity, the roles of several repair (uvrA, recN and oxyR) and recombination (recB, recJ and recO) genes in the response of Escherichia coli to gamma-radiation were studied. The results indicate that all the repair- and recombination-deficient strains were more sensitive to the lethal effects of ionizing radiation. However, the SOS activation pattern was somewhat different. The minimal inducing dose in uvrA and recN mutants was lower than in the wild-type, whereas their SOS response was higher at all doses. Conversely, in the strains lacking an active recB, recJ or recO gene, the doubling dose was almost the same as in the wild-type but the level of induction remained stable over a wide dose range. These findings suggest that neither single- nor double-strand breaks are in

themselves direct SOS inducers and that while uvrA, recN and oxyR take part in different repair or protective pathways, apparently recB, recJ and recO participate in damage processing leading to SOS induction, as well as in recombination repair.

Bucio L, Garcia C, Souza V, Hernandez E, Gonzalez C, Betancourt M, Gutierrez-Ruiz MC. **Uptake, cellular distribution and DNA damage produced by mercuric chloride in a human fetal hepatic cell line.** Mutat Res 1999;423(1-2):65-72.
A human hepatic cell line (WRL-68 cells) was employed to investigate the uptake of the toxic heavy metal mercury. Hg accumulation in WRL-68 cells is a time and concentration dependent process. A rapid initial phase of uptake was followed by a second slower phase. The transport does not require energy and at low HgCl2 concentrations (<50 microM) Hg transport occurs by temperature-insensitive processes. Subcellular distribution of Hg was: 48% in mitochondria, 38% in nucleus and only 8% in cytosolic fraction and 7% in microsomes. Little is known at the molecular level concerning the genotoxic effects following the acute exposure of eucaryotic cells to low concentrations of Hg. Our results showed that Hg induced DNA single-strand breaks or alkali labile sites using the single-cell gel electrophoresis assay (Comet assay). The percentage of damaged nucleus and the average length of DNA migration increased as metal concentration and time exposure increased. Lipid peroxidation, determined as malondialdehyde production in the presence of thiobarbituric acid, followed the same tendency, increased as HgCl2 concentration and time of exposure increased. DNA damage recovery took 8 h after partial metal removed with PBS-EGTA. Copyright 1999 Elsevier Science B.V.

Chen T, Aidoo A, Mittelstaedt RA, Casciano DA, Heflich RH. **Hprt mutant frequency and molecular analysis of Hprt mutations in Fischer 344 rats treated with thiotepa.** Carcinogenesis 1999;20(2):269-77.
Thiotepa is a bifunctional alkylating anticancer drug that is a rodent carcinogen and a suspected human carcinogen. In order to determine the sensitivity of mutant induction in the Hprt lymphocyte assay for detecting tumorigenic doses of thiotepa, Fischer 344 rats were treated for 4 weeks with thiotepa using a procedure adapted from a carcinogenesis protocol. At various times after beginning the treatment regimen, rats were killed and the lymphocyte Hprt assay was performed on splenic lymphocytes isolated from the animals. The 6-thioguanine-resistant T lymphocyte mutant frequency increased with time during the period of thiotepa exposure and declined slightly thereafter. Significant dose-dependent increases in mutant frequency were found using concentrations of thiotepa that eventually result in lymphoproliferative tumors. Hprt mRNA from mutant lymphocytes was reverse transcribed to cDNA, amplified by PCR and examined for mutations by DNA sequencing. This analysis indicated that the major type of point mutation was G:C-->T:A transversion and that 33% of the mutants contained simple or complex frameshifts. Also, a multiplex PCR performed on DNA from mutant clones that were expanded in vitro indicated that 34% of the clones had deletions in the Hprt gene. These results indicate that the induction of lymphocyte Hprt mutants is a sensitive biomarker for the carcinogenicity of thiotepa and that the types of mutations found in the lymphocyte Hprt gene reflect the kinds of DNA damage produced by thiotepa.

Ciaccio PJ, Gicquel E, O'Neill PJ, Scribner HE, Vandenberghe YL. **Investigation of the positive response of $_{43}$ethyl acrylate in the mouse lymphoma genotoxicity assay.** Toxicol Sci 1998;46(2):324-

32.
To develop a better understanding of the relationship between ethyl acrylate (EA)-induced cytotoxicity and mutation frequency in the mouse lymphoma assay (MLA) we measured the effects of EA treatment to ML cells on: (1) nonprotein sulfhydryl (NPS) levels; (2) mitochondrial rhodamine 123 (Rh123) uptake; (3) the DNA elution slope (single-strand breakage) and Y intercept of fitted curves (cytotoxicity and double-strand breakage) in the alkaline elution assay; (4) the appearance of apoptosis; and (5) the pulsed-field gel electrophoretic resolution of high-molecular-weight DNA. EA reduced NPS in both a time- and concentration-dependent manner. By 30 min, > or = 20 micrograms/ml EA reduced NPS by 50% or greater. By 4 h, > or = 10 micrograms/ml markedly decreased both NPS cell content (> or = 71.5% reduction) and mitochondrial Rh123 uptake (10-50 micrograms/ml; 9-62%), the latter effect being further enhanced by washing and incubation for an additional 2 h (12-85%). EA did not induce single-strand breaks in the alkaline elution assay. Only highly cytotoxic EA concentrations (80-87% reduction in RCG at 40-50 micrograms/ml) caused both increases in the elution slope and parallel drops (Y intercept) in the elution curve in the alkaline elution assay. Conventional agarose gel electrophoretic analysis of the DNA neutral fraction of these high dose preparations showed evidence for both apoptosis (180-bp oligonucleosomal DNA laddering effect) and random smearing of DNA (necrosis). Pulsed-field gel electrophoresis of directly loaded high dose cell preparations revealed both high- and low-molecular-weight DNA double-strand breaks, but only at the highest concentrations. These observations indicated that the EA-induced mutagenic response correlated best with cellular cytotoxicity mediated by NPS depletion and mitochondrial membrane impairment.

Cicchetti R, Bari M, Argentin G. **Induction of micronuclei in bone marrow by two pesticides and their differentiation with CREST staining: an in vivo study in mice.** Mutat Res 1999;439(2):239-48.
Two pesticides, organophosphate phosphamidon (PHO) and organochlorine dieldrin (DED) were assayed by the mouse bone marrow micronucleus test, to ascertain whether they showed genotoxic activity in vivo. Two doses, sub-lethal (PHO=3 mg/kg b.wt.; DED=60 mg/kg b.wt.) and lethal (PHO=5 mg/kg b.wt.; DED=90 mg/kg b.wt.), of each substance were administered intraperitoneally to 9-10-week old CBA male mice, in acute and repeated exposure. The sub-lethal dose was also administered at two different times and twice at 24-h intervals. Both PHO and DED proved able to induce a dose-dependent increase of micronucleated polychromatic erythrocytes (PCE). The two pesticides also showed a different detoxification time. Furthermore, the CREST staining with antikinetochore antibodies allowed us to conclude that the two chemicals are clastogens. Copyright 1999 Elsevier Science B.V.

Corran AJ, Renwick A, Dunbar SJ. **Approaches to in-vitro lead generation for fungicide invention.** Pesticide Sci 1998;54(4):338-44.
BIOSIS COPYRIGHT: BIOL ABS. There are increasing opportunities for the development of highthroughput in-vitro screens to aid the discovery of fungicides with novel modes of action. In the past, such screens were developed when biochemical targets were validated by fungicides with defined modes of action. However, genetic information is beginning to have a major impact both on the way in-vitro targets are selected and on the speed at which mode-of-action information is gained on current fungicides having an, as yet, undefined mode of action. This paper discusses issues concerning target selection and high-throughput screening, using examples taken from the current literature and from investigations at Zeneca Agrochemicals, using inhibition of fungal respiration as an example.

Saccharomyces cerevisiae is discussed as model for fungicide research, both in terms of its sensitivity to known fungicides and its well defined molecular genetics, which makes it amenable to such techniques as gene dosage for mode of action determination.

Dean SW, Brooks TM, Burlinson B, Mirsalis J, Myhr B, Recio L, Thybaud V. **Transgenic mouse mutation assay systems can play an important role in regulatory mutagenicity testing in vivo for the detection of site-of-contact mutagens.** Mutagenesis 1999;14(1):141-51.
BIOSIS COPYRIGHT: BIOL ABS. Transgenic mouse mutations assays, such as MutaTMMouse (lacZ, CD2F1) and Big Blue (lacI, B6C3F1), afford the opportunity to evaluate the mutagenic potential of chemicals in any target organ in vivo. This paper discusses published data collected from the analysis of the skin, stomach and lung DNA after topical, oral and inhalation exposure, respectively. These data indicate that both MutaTMMouse and Big Blue should play an important part in the evaluation of genotoxicity in vivo, particularly where the endpoint or target tissue available in the more conventional tests is inappropriate. It is concluded that there is a distinct role for this type of assay in genetic toxicology testing. For substances applied to the skin or dosed orally or by inhalation and which are unlikely to reach either the bone marrow or the liver, then data derived from these assays may be more relevant to an assessment of possible risk to man than the currently used unscheduled DNA synthesis in liver and cytogenetics assays in bone marrow or peripheral blood.

Deptala A, Bedner E, Gorczyca W, Darzynkiewicz Z. **Activation of nuclear factor kappa B (NF-kappaB) assayed by laser scanning cytometry (LSC).** Cytometry 1998;33(3):376-82.
Nuclear factor kappa B (NF-kappaB)/rel is the family of ubiquitous transcriptional activators involved in regulation of diverse immune and inflammatory responses. It also plays a role in control of cell growth and apoptosis. In its inactive form NF-kappaB remains in the cytoplasm sequestered through interaction with IkappaB protein. Rapid translocation of NF-kappaB from cytoplasm to nucleus that occurs in response to extracellular signals is considered to be a hallmark feature of its activation. The translocation of NF-kappaB in HL-60, U-937 and Jurkat leukemic cells as well as in human fibroblasts induced by tumor necrosis factor alpha (TNF-alpha) or phorbol myristate acetate (PMA) was presently measured by laser scanning cytometry (LSC). NF-kappaB was detected immunocytochemically with FITC-tagged antibody and its presence in the nucleus vis-a-vis cytoplasm was monitored by measuring the green fluorescence integrated over the nucleus, which was counterstained with propidium iodide (PI), and over the cytoplasm, respectively. Activation of NF-kappaB led to a rapid increase in NF-kappaB-associated fluorescence measured over the nucleus (FN) concomitant with a decrease in fluorescence over the cytoplasm (F(C)), which was reflected by an increase in F(N)/F(C) ratio. This rapid assay of NF-kappaB activation can be combined with morphological identification of the activated cells or with their immunophenotype. Bivariate analysis of NF-kappaB expression versus cellular DNA content makes it possible to correlate its activation with the cell cycle position. The described method has a potential to be used as a functional assay to monitor intracellular translocation of other transcriptional activators such as p53 tumor suppressor protein or signal transduction molecules.

Dianovsky J, Sivikova K. **Sister chromatid exchanges in sheep peripheral lymphocytes after in vitro exposure to metal- containing emission.** Acta Vet Brno 1998;67(3):183-8.
BIOSIS COPYRIGHT: BIOL ABS. Industrial pollutants, originated from aluminum processing factory,

were tested for induction of sister chromatid exchanges (SCE). Experiments were carried out using sheep peripheral lymphocytes under in vitro assays. The emission tested contained Al, Cd, As, Mn, Pb, Cu, Zn, Fe, Na, Ca, and Mg; the majority of them in the form of sulphides, sulphates, oxides or fluorides. The emission was dissolved and neutralized according to a standard method. The mean of SCE was determined for three concentrations the emitted material (30, 60 and 90 mug /ml) with presence and absence of the metabolic activation (S9 mix). The lowest concentration used corresponded to daily oral intake by sheep grazing on contaminated area. Results from both assays were similar; the significant increase in SCE was observed at the concentration of 60 mug/ml ($p < 0.01$). More conspicuous results were observed without of S 9. No significant decrease in the induction of proliferation index (PI) was found. A dose related effect was observed for SCE induction but not for inhibition of proliferation kinetics.

Dobrovolsky VN, Casciano DA, Heflich RH. **Tk+/- mouse model for detecting in vivo mutation in an endogenous, autosomal gene.** Mutat Res 1999;423(1-2):125-36.
Tk+/- transgenic mice were created using an embryonic stem cell line in which one allele of the endogenous thymidine kinase (Tk) gene was inactivated by targeted homologous recombination. Breeding Tk+/- parents produced viable Tk-/- knockout (KO) mice. Splenic lymphocytes from KO mice were used in reconstruction experiments for determining the conditions necessary for recovering Tk somatic cell mutants from Tk+/- mice. The cloning efficiency of KO lymphocytes was not affected by the toxic thymidine analogues 5-bromo-2'-deoxyuridine (BrdUrd) or trifluorothymidine (TFT), or by BrdUrd in the presence of lymphocytes from Tk+/- animals; however, it was easier to identify clones resistant to BrdUrd than to TFT when Tk+/- cells were present. Tk+/- mice were treated with vehicle or 100 mg/kg of N-ethyl-N-nitrosourea (ENU), and after 4 months, the frequency of Tk mutant lymphocytes was measured by resistance to BrdUrd. The frequency of Tk mutants was 22+/-5.9x10-6 in control animals and 80+/-31x10-6 in treated mice. In comparison, the frequency of Hprt mutant lymphocytes, as measured by resistance to 6-thioguanine, was 2.0+/-1.2x10-6 in control animals and 84 +/-28x10-6 in the ENU-treated mice. Analysis of BrdUrd-resistant lymphocyte clones derived from the ENU-treated animals revealed point mutations in the non-targeted Tk allele. These results indicate that the selection of BrdUrd-resistant lymphocytes from Tk+/- mice may be used for assessing in vivo mutation in an endogenous, autosomal gene.

Eastham AM, Marples B, Kiltie AE, Orton CJ, West CM. **Fibroblast radiosensitivity measured using the comet DNA-damage assay correlates with clonogenic survival parameters.** Br J Cancer 1999;79 (9-10):1366-71.
A study was made of the neutral comet assay as a potential method for measuring normal cell radiosensitivity. Eleven fibroblast strains were studied comprising nine derived from vaginal biopsies from pretreatment cervical cancer patients and two strains from radiosensitive individuals. DNA double strand break (dsbs) dose-response curves for both initial and residual (20-h repair time) damage were obtained over the dose range 0-240 Gy, with slopes varying 3.2 and 8-fold respectively. Clonogenic cell survival parameters were available for all the cell strains following both high- and low-dose rate irradiation. There were no correlations between the dose-response slope of the initial level of DNA dsbs and parameters that mainly describe the initial portion of clonogenic radiation survival curves (SF2, alpha, D). A significant correlation ($r = -0.63$, $P = 0.04$) was found between the extent of residual DNA

dsbs and clonogenicity for all 11 fibroblast strains. The parameter showing the highest correlation with fibroblast cell killing (D) for the nine normal fibroblasts alone was the ratio of initial/residual DNA dsb dose-response slope (r = 0.80, P = < 0.01). A significant correlation (r = -0.67, P = 0.03) with clonogenic radiosensitivity was also found for all 11 cell strains when using the ratio of initial/residual DNA dsb damage at a single dose of 180 Gy. This study shows that fibroblast radiosensitivity measured using the neutral comet assay correlates with clonogenic radiation survival parameters, and therefore may have potential value in predictive testing of normal tissue radiosensitivity.

Eckert KA, Hile SE. **Alkylation-induced frameshift mutagenesis during in vitro DNA synthesis by DNA polymerases alpha and beta.** Mutat Res 1998;422(2):255-69.
We have analyzed the mutational spectra produced during in vitro DNA synthesis by DNA polymerase alpha-primase and DNA polymerase beta. The polymerase mutation frequency as measured in the in vitro herpes simplex virus thymidine kinase (HSV-tk) forward assay was increased when reactions utilized single-stranded DNA templates randomly modified by 20 mM N-ethyl-N-nitrosourea (ENU), relative to solvent-treated templates. A 20- to 50-fold increase in the frequency of G-->A transition mutations was observed for both polymerases, as expected due to mispairing by O6-ethylguanine lesions. Strikingly, ENU treatment of the template also resulted in a five- to 12-fold increased frequency of frameshift errors at heteropolymeric (non-repetitive) template sequences produced by polymerase beta and polymerase alpha-primase, respectively. The increased proportion of frameshift mutations at heteropolymeric sequences relative to homopolymeric (repetitive) sequences produced by each polymerase in response to ENU damage was statistically significant. For polymerase alpha-primase, one-base deletion errors at template guanine residues was the second most frequent mutational event, observed at a frequency only four-fold lower than the G-->A transition frequency. In the polymerase beta reactions, the frequency of insertion errors at homopolymeric (repetitive) sequences was increased six-fold using alkylated templates, relative to solvent controls. The frequency of such insertion errors was only three-fold lower than the frequency of G-->A transition errors by polymerase beta. Although ENU is generally regarded as a potent base substitution mutagen, these data show that monofunctional alkylating agents are capable of inducing frameshift mutations in vitro. Alkylation-induced frameshift mutations occur in both repetitive and non-repetitive DNA sequences; however, the mutational specificity is dependent upon the DNA polymerase. Copyright 1998 Elsevier Science B. V.

Eckert KA, Opresko PL. **DNA polymerase mutagenic bypass and proofreading of endogenous DNA lesions.** Mutat Res 1999;424(1-2):221-36.
DNA polymerases differentiate between correct and incorrect substrates during synthesis on undamaged DNA templates through the biochemical steps of base incorporation, primer-template extension and proofreading excision. Recent research examining DNA polymerase processing of abasic, alkylation and oxidative lesions is reviewed in light of these discrimination mechanisms. Inhibition of DNA synthesis results from correct polymerase discrimination against utilization of geometrically incorrect template bases or 3' terminal basepairs. The efficiency of translesion synthesis is thus related to the physical structure of the lesion containing DNA. However, variations in enzyme structure and kinetics result in translesion synthesis efficiencies that are also dependent upon the DNA polymerase. With a low probability, polymerase misinsertion events create a 3' lesion terminus which is geometrically favored over the correct lesion basepair, resulting in mutagenic translesion synthesis. For example, both

polymerase alpha and polymerase beta appear to require the formation of a stable 3' primer-template structure for efficient abasic site translesion synthesis. However, the enzymes differ as to the precise molecular make-up of the stable DNA structure, resulting in different mutational specificities. Similar mechanisms may be applicable to oxidative damage, where mutational specificities dependent upon the DNA polymerase also have been observed. In vitro reaction conditions also influence DNA polymerase processing of lesions. Using an in vitro herpes simplex virus thymidine kinase (HSV-tk) gene forward mutation assay, we demonstrate that high dNTP substrate concentrations affect the mutagenic specificity of translesion synthesis using alkylated templates. The exonuclease-deficient Klenow polymerase error frequency for G-->A transition mutations using templates modified by N-ethyl-N-nitrosourea (ENU) was four-fold higher at 1000 microM [dNTP], relative to 50 microM [dNTP], consistent with an increased efficiency of extension of the etO6G.T mispair. Moreover, the frequency of other ENU-induced polymerase errors was suppressed when polymerase reactions contained 50 microM dNTP, relative to 1000 microM dNTP. The efficiency of proofreading as a polymerase error discrimination mechanism reflects a balance between the competing processes of 3'-->5' exonuclease removal of mispairs and polymerization of the next correct nucleotide. Polymerases that are devoid of a proofreading exonuclease generally display enhanced abasic site translesion synthesis relative to proofreading-proficient enzymes. In addition, the proofreading exonucleases of Escherichia coli Pol I and T4 DNA polymerases have been found to remove mispairs caused by abasic sites and oxidative lesions, respectively, resulting in lowered polymerase error rates. However, the magnitude of the exonuclease effect is small (less than 10-fold), and highly dependent upon the DNA polymerase-exonuclease. We have studied proofreading exonuclease removal of alkylation damage in the HSV-tk forward assay. We observed no significant reduction in the magnitude of the mutant frequency vs. dose-response curves when N-methyl-N-nitrosourea or ENU-treated templates were used in exonuclease-proficient Klenow polymerase reactions, as compared to the exonuclease-deficient polymerase reactions. Thus, available data suggest that proofreading excision of endogenous lesion mispairs does occur, but the efficiency is dependent upon the lesion and the DNA polymerase-exonuclease studied. Copyright 1999 Elsevier Science B.V.

Ember I, Kiss I, Gombkoto G, Muller E, Szeremi M. **Oncogene and suppressor gene expression as a biomarker for ethylene oxide exposure.** Cancer Detect Prev 1998;22(3):241-5.
Ethylene oxide is a proven genotoxic chemical, and there is lots of evidence suggesting its carcinogenic effects in humans. The unexpected massive appearance of a certain tumorous cluster in personnel exposed to ethylene oxide in a Hungarian county hospital focused attention on the effects of this toxic gas. Since we had developed an animal model for the investigation of alterations in onco/suppressor gene expression due to external carcinogenic agents, and this model had already been used to evaluate the carcinogenic effects of cytostatic drugs in humans, an analysis of the effects of ethylene oxide exposure seemed to offer further information on the usefulness of gene expression as a biomarker. The main purpose of our study was to determine whether or not ethylene oxide exposure causes an elevated expression of onco/suppressor genes in the white blood cells of exposed people. Two different exposed groups and one control group were included in the study. The N-ras and p53 genes were chosen for the investigations of gene expression. N-ras is known to be activated in several tumor types, and p53 is also involved in carcinogenesis and plays an important role in the cellular answer mechanism to exogenous toxic effects. RNA was isolated from the white blood cells, slot blotted onto nitrocellulose membranes,

and hybridized with chemoluminescently labeled gene probes. The results were detected on X-ray films and scanned into a computer, and relative risk for elevated gene expression was calculated in each group. Elevated N-ras and detectable p53 expressions were observed more frequently in both exposed groups compared with the control group (relative risks--N-ras: 1.57 [0.77-3.22] and 2.34 [1.21-4.52]; p53: 6.67 [2.35-18.92] and 6.06 [2.10-17.49]).

Gaivao I, Sierra LM, Comendador MA. **The w/w+ SMART assay of Drosophila melanogaster detects the genotoxic effects of reactive oxygen species inducing compounds.** Mutat Res 1999;440(2):139-45.
The somatic mutation and recombination w/w+ eye assay has been used for genotoxic evaluation of a broad number of chemicals with different action mechanisms yielding high values of sensitivity, specificity and accuracy. The aim of this work was to determine the utility of this assay in the evaluation of reactive oxygen species inducers. For this, we have tested eight compounds: diquat, paraquat, menadione, juglone, plumbagin, streptonigrin, tert-butyl hydroperoxide and 4-nitroquinoline 1-oxide, using the Drosophila Oregon K strain which had previously shown advantageous conditions to test this type of compounds. Diquat was the only chemical for which the results were clearly negative, probably because its high toxicity, whereas indications of a marginal genotoxicity raised for menadione. The remaining compounds were evaluated as positives. The conclusion of these experiments is that the w/w+ assay is capable to detect genotoxic effects induced by compounds that generate reactive oxygen species through different action mechanisms. Copyright 1999 Elsevier Science B.V.

Garsin DA, Paskowitz DM, Duncan L, Losick R. **Evidence for common sites of contact between the antisigma factor SpoIIAB and its partners SpoIIAA and the developmental transcription factor sigmaF in Bacillus subtilis.** J Mol Biol 1998;284(3):557-68.
The activity of the developmental transcription factor sigmaF in Bacillus subtilis is governed by a switch involving the dual function protein SpoIIAB. SpoIIAB is an antisigma factor that forms complexes with sigmaF and with an alternative partner protein SpoIIAA. SpoIIAB is also a protein kinase that can inactivate SpoIIAA by phosphorylating it on a serine residue. We sought to identify amino acids in SpoIIAB that are involved in the formation of the SpoIIAB-SpoIIAA complex by screening for mutants that were defective in the activation of sigmaF. This genetic screen, in combination with biochemical analysis and the construction of loss-of-side-chain (alanine substitution) mutants, led to the identification of amino acid side-chains in the N-terminal region of SpoIIAB that could contact SpoIIAA. Unexpectedly, the same amino acid side-chains (R20 and N50) that appear to touch SpoIIAA are required for binding to, and may represent sites of contact with, sigmaF. We propose that the N-terminal region of SpoIIAB forms a binding surface that is responsible for the formation of both the SpoIIAB-SpoIIAA and the SpoIIAB-sigmaF complexes, and that in some cases the same amino acid side-chains contact both partner proteins. N50 is also the defining residue of a region of amino acid sequence homology known as the N-box that is shared by SpoIIAB and related serine protein kinases, as well as by members of a mechanistically dissimilar family of protein kinases that undergo autophosphorylation at a histidine residue. We discuss the implications of this finding for the mechanism of histidine autophosphorylation. Copyright 1998 Academic Press.

Guillaume-Gable C, Floch V, Mercier B, Audrezet MP, Gobin E, Le Bolch G, Yaouanc JJ, Clement JC,

Des Abbayes H, Leroy JP, et al. **Cationic phosphonolipids as nonviral gene transfer agents in the lungs of mice.** Hum Gene Ther 1998;9(16):2309-19.
With the aim of developing new gene transfer tools for treating CF with gene therapy, we have synthesized a novel family of molecules named cationic phosphonolipids. The most efficient among them were selected by in vitro screening to compare their activities in vivo in mouse lungs. We used a reporter gene whose activity was measured cytofluorimetrically (FACS-Gal assay) and by means of a chemiluminescence technique. These tests allowed us to identify the percentage of transfected cells and to quantify total beta-galactosidase in the lungs. This enabled us to identify two molecules, significantly efficient in comparison with DNA alone: GLB73 ($p = 0.0015$) and GLB253 ($p = 0.007$). Their use resulted in a time lag between transfection and maximum efficiency: maximum efficiency was observed 4 days after transfection with GLB73, whereas it was noticeable only on day 7 with GLB253. Moreover, from toxicity studies carried out in vivo, GLB73 seems to be nontoxic. In vivo results were correlated with in vitro results obtained with CF epithelial cell lines. Consequently, GLB73 is a potential candidate for phase I clinical trials in humans.

Hill KA, Nishino H, Buettner VL, Halangoda A, Li W, Sommer SS. **The Big Blue(R) transgenic mouse mutation detection assay: the mutation pattern of sectored mutant plaques.** Mutat Res 1999;425(1):47-54.
There are mutational artifacts in the Big Blue(R) assay and it is important to characterize the source and nature of these mutations. Differences were reported in the mutation patterns of a small sample of 23 sectored and 91 circular mutant plaques derived from skin using the Big Blue(R) transgenic mouse mutation detection system [G. R. Stuart, N.J. Gorelick, J.L. Andrews, J.G. de Boer, B.W. Glickman, The genetic analysis of lacI mutations in sectored plaques from Big Blue transgenic mice, Environ. Mol. Mutagen 28 (1996) 385-392.]. We have extended these observations by analyzing 46 sectored and 224 circular mutant plaques derived from seven tissues. The frequency of sectored mutant plaques is estimated to be 16% with no significant variation with tissue type. However, the patterns of mutation for sectored mutants and mouse-derived mutations differed significantly ($p=0.04$). Base substitutions in sectored mutant plaques do not show the asymmetries found in circular mutants consistent with integration of a GC rich transgene into the AT rich mammalian genome. Sectored mutants have mutation patterns consistent with a mixture of mouse, in vitro and Escherichia coli-derived mutations. Data on the relative frequencies of different mutant plaque morphologies suggests that overlapped plaques are substantially contaminated by sectored plaques at recommended plating densities. Copyright 1999 Elsevier Science B.V.

Holt JR, Johns DC, Wang S, Chen ZY, Dunn RJ, Marban E, Corey DP. **Functional expression of exogenous proteins in mammalian sensory hair cells infected with adenoviral vectors.** J Neurophysiol 1999;81(4):1881-8.
To understand the function of specific proteins in sensory hair cells, it is necessary to add or inactivate those proteins in a system where their physiological effects can be studied. Unfortunately, the usefulness of heterologous expression systems for the study of many hair cell proteins is limited by the inherent difficulty of reconstituting the hair cell's exquisite cytoarchitecture. Expression of exogenous proteins within hair cells themselves may provide an alternative approach. Because recombinant viruses were efficient vectors for gene delivery in other systems, we screened three viral vectors for their ability to

express exogenous genes in hair cells of organotypic cultures from mouse auditory and vestibular organs. We observed no expression of the genes for beta-galactosidase or green fluorescent protein (GFP) with either herpes simplex virus or adeno-associated virus. On the other hand, we found robust expression of GFP in hair cells exposed to a recombinant, replication-deficient adenovirus that carried the gene for GFP driven by a cytomegalovirus promoter. Titers of 4 x 10(7) pfu/ml were sufficient for expression in 50% of the approximately 1,000 hair cells in the utricular epithelium; < 1% of the nonhair cells in the epithelium were GFP positive. Expression of GFP was evident as early as 12 h postinfection, was maximal at 4 days, and continued for at least 10 days. Over the first 36 h there was no evidence of toxicity. We recorded normal voltage-dependent and transduction currents from infected cells identified by GFP fluorescence. At longer times hair bundle integrity was compromised despite a cell body that appeared healthy. To assess the ability of adenovirus-mediated gene transfer to alter hair cell function we introduced the gene for the ion channel Kir2.1. We used an adenovirus vector encoding Kir2.1 fused to GFP under the control of an ecdysone promoter. Unlike the diffuse distribution within the cell body we observed with GFP, the ion channel-GFP fusion showed a pattern of fluorescence that was restricted to the cell membrane and a few extranuclear punctate regions. Patch-clamp recordings confirmed the expression of an inward rectifier with a conductance of 43 nS, over an order of magnitude larger than the endogenous inward rectifier. The zero-current potential in infected cells was shifted by -17 mV. These results demonstrate an efficient method for gene transfer into both vestibular and auditory hair cells in culture, which can be used to study the effects of gene products on hair cell function.

Honma M, Hayashi M, Shimada H, Tanaka N, Wakuri S, Awogi T, Yamamoto KI, Kodani NU, Nishi Y, Nakadate M, et al. **Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the in vitro chromosomal aberration test.** Mutagenesis 1999;14(1):5-22.
BIOSIS COPYRIGHT: BIOL ABS. In order to evaluate the utility of the mouse lymphoma assay (MLA) for detecting in vitro clastogens and spindle poisons and to compare it with the in vitro chromosomal aberration test (CA), we conducted an international collaborative study of the MLA that included 45 Japanese laboratories and seven overseas laboratories under the cooperation of the Ministry of Health and Welfare of Japan and the Japanese Pharmaceutical Manufacturer's Association. We examined 40 chemicals; 33 were reportedly positive in the CA but negative in the bacterial reverse mutation assay, six were negative in both assays and one was positive in both. We assayed mutations of the thymidine kinase (TK) locus (tk) of L5178Y tk+/- mouse lymphoma cells using the microwell method. According to our standard protocol, cells were exposed to the chemical for 3 h, cultured for 2 days and TK-deficient mutants were expressed in 96-well plates under trifluorothymidine. Each chemical was coded and tested by two or three laboratories. Among the 34 CA-positive chemicals, positive MLA results were obtained for 20 and negative results were obtained for nine. The remaining five chemicals were inconclusive or equivocal because of discrepant inter-laboratory results or reproduced discrepant results, respectively. Among the six CA-negative chemicals, one was negative in the MLA, two were positive and three were inconclusive. Thus, the MLA could detect only 59% (20/34) of CA-positive chemicals. We concluded that the MLA was not as sensitive as the CA. Some MLA-negative chemicals evoked positive responses in the CA only after long continuous treatment. These might also be genotoxic in the MLA with long continuous treatment. Improvement of the MLA protocol, including alteration of the duration of the treatment, might render the MLA as sensitive as the CA.

Jaloszynski P, Kujawski M, Wasowicz M, Szulc R, Szyfter K. **Genotoxicity of inhalation anesthetics halothane and isoflurane in human lymphocytes studied in vitro using the comet assay.** Mutat Res 1999;439(2):199-206.
The alkaline single cell gel electrophoresis (comet) assay was applied to study genotoxic properties of two inhalation anesthetics-halothane and isoflurane-in human peripheral blood lymphocytes (PBL). The cells were exposed in vitro to either halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) or isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) at concentrations 0.1-10 mM in DMSO. The anesthetics-induced DNA strand breaks as well as alkali-labile sites were measured as total comet length (i.e., increase of a DNA migration). Both analysed drugs were capable of increasing DNA migration in a dose-dependent manner. In experiments conducted at two different electrophoretic conditions (0. 56 and 0.78 V/cm), halothane was able to increase DNA migration to a higher extent than isoflurane. The comet assay detects DNA strand breaks induced directly by genotoxic agents as well as DNA degradation due to cell death. For this reason a contribution of toxicity in the observed effects was examined. We tested whether the exposed PBL were able to repair halothane- and isoflurane-induced DNA damage. The treated cells were incubated in a drug-free medium at 37 degrees C for 120 min to allow processing of the induced DNA damage. PBL exposed to isoflurane at 1 mM were able to complete repair within 60 min whereas for halothane a similar result was obtained at a concentration lower by one order of magnitude: the cells exposed to halothane at 1 mM removed the damage within 120 min only partly. We conclude that the increase of DNA migration induced in PBL by isoflurane at 1 mM and by halothane at 0.1 mM was not a result of cell death-associated DNA degradation but was caused by genotoxic action of the drugs. The DNA damage detected after the exposure to halothane at 1 mM was in part a result of DNA fragmentation due to cell death. Copyright 1999 Elsevier Science B.V.

Kaneko M, Inoue F. **The sensitivity to DNA single strand breakage in mitochondria, but not in nuclei, of Chinese hamster V79 and variant cells correlates with their cellular sensitivity to hydrogen peroxide.** Toxicol Lett 1998;99(1):15-22.
To investigate whether a relation exists between the level of DNA damage by and cytotoxicity of hydrogen peroxide, we measured the initial level of H2O2-induced nuclear and mitochondrial DNA single strand breaks in Chinese hamster V79 and H2O2-resistant variant cells (Hpr-4) with an alkaline elution technique and a quantitative Southern blot technique, respectively. The frequency of DNA single strand breaks in mitochondrial DNA induced by H2O2 was more than one hundred times that of nuclear DNA in the parent V79 cells. While a similar frequency of nuclear DNA single strand breaks was generated in V79 and Hpr-4 cells at an equidose of H2O2, a lower number of mitochondrial DNA single strand breaks were generated in Hpr-4 cells than in V79 cells by H2O2 in the range of 100 microM to 5 mM. The sensitivity to mitochondrial DNA single strand break-induction correlated with the cellular sensitivity to H2O2 in Chinese hamster V79 and variant cells.

Kasai Y, Stahl S, Crews S. **Specification of the Drosophila CNS midline cell lineage: direct control of single-minded transcription by dorsal/ventral patterning genes.** Gene Expr 1998;7(3):171-89.
The Drosophila CNS consists of a bilaterally symmetric group of neurons separated by a discrete group of CNS midline cells. The specification of the CNS midline cell lineage requires transcription of the single-minded gene. Genetic evidence suggests that a group of transcription factors, including Dorsal, Snail, Twist, and Daughterless:: Scute, is required for initial single-minded transcription. Comparison of

the DNA sequences of the single-minded gene regulatory regions between two Drosophila species reveals conserved sequence elements. Biochemical studies using purified proteins indicate that a number of these conserved sequences represent binding sites for Dorsal, Snail, and Twist. In vitro mutagenesis combined with germline transformation indicates that these binding sites are required in vivo for single-minded mesectodermal transcription. These results show that single-minded transcription and, thus, CNS midline specification is directly controlled by dorsal/ventral patterning transcription factors. They also suggest a model in which multiple transcriptional activators function in a cooperative, concentration-dependent mode in combination with a transcriptional repressor to restrict single-minded transcription to the CNS midline precursor cells.

Kawakami K, Koga A, Hori H, Shima A. **Excision of the tol2 transposable element of the medaka fish, Oryzias latipes, in zebrafish, Danio rerio.** Gene 1999;225(1-2):17-22.
The Tol2 element is a transposable element in Oryzias latipes (the medaka fish) found in the tyrosinase gene locus of the tyrosinase-deficient mutant medaka fish and has been shown to be excised from the genome during medaka embryogenesis (Koga, A., Suzuki, M., Inagaki, H., Bessho, Y., Hori, H., 1996. Transposon element in fish. Nature 383, 30). It is, however, not known whether the Tol2 element is an autonomous element. To determine whether the cloned Tol2 element is an autonomous element and whether excision can occur also in the other fish species, the plasmid DNA harboring the Tol2 element was injected to fertilized eggs of zebrafish, Danio rerio, and the total DNA extracted from the embryos 9-10h after the injection was analyzed by PCR. When a plasmid with the full-length Tol2 element was used for the microinjection, in 39 out of 43 injected embryos, we found generation of short PCR products indicative of the loss of the Tol2 element from the injected plasmid. Ten of these cases were analyzed at the DNA sequence level, and nine of them showed either precise excision of the Tol2 element (three cases) or nearly precise excision of the element with the addition of a few nucleotides of the target duplication (six cases). When a deletion version of the Tol2 element that retained the terminal inverted repeats but lacked about one-fourth of the open reading frame-coding region was used for the microinjection, such short PCR products could not be amplified from any of the injected embryos (0 out of 30). Thus, the Tol2 element is capable of excision in zebrafish embryos, presumably dependent on a putative transposase encoded by the Tol2 element itself. This transient embryonic excision assay using zebrafish should be useful to analyze the structure and the function of the transposase and cis-elements necessary for excision. Also, this study implies the potential use of the Tol2 element in transgenesis and insertional mutagenesis in both zebrafish and the medaka fish.

Khaitovich P, Tenson T, Kloss P, Mankin AS. **Reconstitution of functionally active Thermus aquaticus large ribosomal subunits with in vitro-transcribed rRNA.** Biochemistry 1999;38(6):1780-8.
Functionally active large ribosomal subunits of thermophilic bacterium Thermus aquaticus have been assembled in vitro from ribosomal proteins and either natural or in vitro-transcribed 23S rRNA and 5S rRNA. Sedimentation properties of reconstituted subunits were similar to those of native ribosomal 50S subunits. Subunits reconstituted with in vitro-transcribed rRNAs exhibited high activity in the peptidyl transferase assay and in a poly(U)-dependent cell-free translation system (22 and 30%, respectively, compared to that of native 50S subunits). Catalytic activity of reconstituted subunits critically depended on the presence of 5S rRNA. rRNA mutations known to affect functions of the native ribosome

produced similar effects in reconstituted T. aquaticus 50S subunits. Subunits assembled with in vitro-transcribed T. aquaticus 23S rRNA containing the G2267A mutation (G2252A in Escherichia coli), which interferes with binding of peptidyl-tRNA in the ribosomal P-site, showed drastically reduced peptidyl transferase activity, whereas clindamycin resistance mutation A2084G (A2058G in E. coli) rendered assembled subunits tolerant to clindamycin inhibition. Thus, reconstitution of functional subunits with in vitro-transcribed rRNA makes possible the use of in vitro genetics for mutational analysis of 23S rRNA functions in translation. In addition, the ability to assemble catalytically active 50S subunits from the rRNA transcript lacking any posttranscriptional modifications clearly demonstrates that modified nucleotides in 23S rRNA are dispensable for the principal activities of the ribosome.

Kim BS, Margolin BH. **Statistical methods for the Ames Salmonella assay: a review.** Mutat Res 1999;436(1):113-22.
The Ames Salmonella assay remains the most widely used in vitro genotoxicity assay. Several statistical methods have been proposed for its analysis [B.H. Margolin, N. Kaplan, E. Zeiger, Statistical analysis of the Ames Salmonella/microsome test, Proc. Natl. Acad. Sci., 78 (1981) 3779-3783; L.E. Myers, N.H. Saxton, L.I. Southerland, T.J. Wolff, Regression analysis of Ames test data, Environ. Mol. Mutagen., 3 (1981) 575-586; A.G. Stead, V. Hasselblad, J.P. Creason, L. Claxton, Modelling the Ames test, Mutation Res., 85 (1981) 13-27; L. Bernstein, J. Kaldor, J. McCaan, M.C. Pike, An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test, Mutation Res., 97 (1982) 267-281; N.E. Breslow, Extra-Poisson variation in log-linear models, Appl. Stat., 33 (1984) 38-44; J. Wahrendorf, G.A.T. Mahon, M. Schumacher, A nonparametric approach to the statistical analysis of mutagenicity data, Mutation Res., 147 (1985) 5-13; D.G. Simpson, B.H. Margolin, Recursive nonparametric testing for dose-response relationships subject to downturns at high doses, Biometrika, 73 (1986) 589-596; D.G. Simpson, B.H. Margolin, Nonparametric testing for dose-response curves subject to downturns: Asymptotic power considerations, Annals Stat., 18 (1990) 373-390.]. In this paper we review recent literature to see what statistical methods are in fact employed for the analysis of the Ames assay. We then note that these methods can be classified into a common category in the framework of Haynes and Eckardt's mutation induction kinetics model [R.H. Haynes, F. Eckardt, Mathematical analysis of mutation induction kinetics, in: F.J. de Serres, A. Hollaender (Eds.), Chemical Mutagens, Principles and Methods for Their Detection, Vol. 6, Plenum, New York, 1980, pp. 271-307]. The value in knowing this is that most methods of analysis considered here will likely exhibit common statistical behavior. These analyses are computationally intensive, e.g., [B.H. Margolin, N. Kaplan, E. Zeiger, Statistical analysis of the Ames Salmonella/microsome test, Proc. Nat. Acad. Sci., 78 (1981) 3779-3783], hence the ready availability of computer programs is essential if biologists are to use these methods. We briefly review two statistical software programs that are available in the public domain, and describe in detail a third program, Salm, [B.H. Margolin, N. Kaplan, E. Zeiger, Statistical analysis of the Ames Salmonella/microsome test, Proc. Nat. Acad. Sci., 78 (1981) 3779-3783; B.H. Margolin, B. S. Kim, K. Risko, The Ames Salmonella/microsome assay: Issues of inference and validation, J. Amer. Stat. Assoc., 84 (1989) 651-661]. The Salm program is obtainable through the file transfer protocol (ftp) or using a WWW browser. Finally, we discuss two statistical consequences of naively applying the two-fold rule, a method of analysis employed by a number of researchers. Copyright 1999 Elsevier Science B.V.

Malachova K. **Mutagenicity tests on the bacteria and the detection of genotoxicity of industrial complex mixtures containing PAHs.** Cent Eur J Public Health 1998;6(4):307-13.
The study summarizes the results of an evaluation of mutagenicity of heterogeneous complex mixtures of substances, the main mutagenic component of which consists of polycyclic aromatic hydrocarbons. The testing was performed using bacterial assays of mutagenicity--the SOS chromotest and the S. typhimurium His-test (in modifications without and with metabolic activation in vitro). It was found that samples of tested tar mixtures (crude tar, pitch, anthracene oils III and II, granulated pitch and some of its extraction portions) induced SOS repair functions and frameshift mutations in tests with metabolic activation. Some of samples as tar, pitch and anthracene oils III, granulated pitch and two its extraction portions--LRAC and LRBe--induced also frameshift mutations, and SOS repairs in tests without the metabolic activation. In one sample--LRBe--the ability to induce mutations in all variants of both tests, was also proved. The evaluation of mutagenicity of fly ashes showed that differences in the mutagenic activity of samples can be directly dependent on the extraction method chosen and on the type of extraction agent used. The study results demonstrate that the bacterial tests Salmonella typhimurium His- and the SOS chromotest are uninterchangeable and quite independent. Both tests can be used for orientative screening for genotoxicity in a wide range of various complex mixtures arising from industrial production and contaminating the environment.

Mayer C, Klein RG, Wesch H, Schmezer P. **Nickel subsulfide is genotoxic in vitro but shows no mutagenic potential in respiratory tract tissues of BigBlue rats and Muta Mouse mice in vivo after inhalation.** Mutat Res 1998;420(1-3):85-98.
Carcinogenic nickel compounds are known to induce promutagenic DNA lesions such as DNA strand breaks and DNA adducts in cultured mammalian cells. In standard mutation assays, in contrast, they were found to be either inactive or weakly active. In our in vitro mutation studies in a lacI transgenic embryonic fibroblast cell line, nickel subsulfide (Ni3S2) increased mutation frequency up to 4. 5-fold. We subsequently applied the comet assay and transgenic rodent mutation assays to investigate the DNA damaging effect and mutagenic potential of nickel subsulfide in target cells of carcinogenesis. A 2-h in vitro treatment of freshly isolated mouse nasal mucosa and lung cells with nickel subsulfide clearly induced DNA fragmentation in a concentration dependent manner. The strong effect was not seen in the same cell types following inhalative treatment of mice and rats, leading only in the mouse nasal mucosa to high DNA damage. When the same inhalative treatment was applied to lacZ and lacI transgenic mice and rats, the spontaneous mutation frequency of these target genes in the respiratory tissues was not increased. These results support a recently proposed non-genotoxic model of nickel carcinogenesis, which acts through gene silencing via DNA methylation and chromatin condensation. This model may also explain our in vitro mutation data in the lacI transgenic cell line, in which nickel subsulfide increased mutation frequency, but in about one-third of the mutants, molecular analysis did not reveal any DNA sequence change in the coding region of the lacI gene despite of the phenotypic loss of its function. Copyright 1998 Elsevier Science B.V.

Mohankumar MN, Paul SF, Venkatachalam P, Jeevanram RK. **Influence of in vitro low-level gamma-radiation on the UV-induced DNA repair capacity of human lymphocytes--analysed by unscheduled DNA synthesis (UDS) and comet assay.** Radiat Environ Biophys 1998;37(4):267-75.

Unscheduled DNA synthesis (UDS) induced by ultraviolet radiation (UV) was studied in human lymphocytes after exposing blood samples in vitro to doses ranging between 1 and 10 mGy gamma-radiation, by way of measuring tritiated thymidine (3H-TdR) uptake in the DNA of these lymphocytes. The results indicate that samples pre-exposed to gamma-ray doses ranging between 2.5 and 4 mGy show higher UDS levels compared with those pre-exposed to doses of less than 2.5 or more than 4 mGy. These results were verified by studying the rate of removal of UV-induced photoproducts using the comet assay. The reason for the increase in DNA repair capacity in this dose range is discussed in comparison with earlier reports on this phenomenon. The DNA repair capacity with respect to inter-individual variability and age is also analysed. The study implies that the comet assay is a simple and sensitive visual method to track nucleotide excision repair and hence can be used to estimate UV-induced DNA repair in the place of the more reliable yet cumbersome and time-consuming, grain-counting autoradiographic technique.

Nuwaysir EF, Bittner M, Trent J, Barrett JC, Afshari CA. **Microarrays and toxicology: the advent of toxicogenomics.** Mol Carcinog 1999;24(3):153-9.
The availability of genome-scale DNA sequence information and reagents has radically altered life-science research. This revolution has led to the development of a new scientific subdiscipline derived from a combination of the fields of toxicology and genomics. This subdiscipline, termed toxicogenomics, is concerned with the identification of potential human and environmental toxicants, and their putative mechanisms of action, through the use of genomics resources. One such resource is DNA microarrays or "chips," which allow the monitoring of the expression levels of thousands of genes simultaneously. Here we propose a general method by which gene expression, as measured by cDNA microarrays, can be used as a highly sensitive and informative marker for toxicity. Our purpose is to acquaint the reader with the development and current state of microarray technology and to present our view of the usefulness of microarrays to the field of toxicology.

Obata F, Nunoshiba T, Hashimoto-Gotoh T, Yamamoto K. **An improved system for selection of forward mutations in an Escherichia coli supF gene carried by plasmids.** J Radiat Res (Tokyo) 1998;39(4):263-70.
An improved system to examine forward mutations that occurred in the supF gene of Escherichia coli carried on a multicopy plasmid is described. The system was validated by measuring spontaneous mutations of supF plasmids propagated in wild-type, recA- and mutM- mutY- E. coli strains, the mutation frequencies of which were 1.3 x 10(-7), 6.3 x 10(-7) and 1.5 x 10(-6), respectively. Sequence analysis of the supF mutant plasmids revealed that G:C-->T:A and G:C-->C:G transversions dominated. This improved system allows rapid scoring and sequencing forward mutations in the supF gene, thus permitting its use as a genetic target for repair and mutagenesis studies in bacteria and mammalian cells.

Park GH, Plummer HK 3rd, Krystal GW. **Selective Sp1 binding is critical for maximal activity of the human c-kit promoter.** Blood 1998;92(11):4138-49.
The receptor tyrosine kinase c-kit is necessary for normal hematopoiesis, the development of germ cells and melanocytes, and the pathogenesis of certain hematologic and nonhematologic malignancies. To better understand the regulation of the c-kit gene, a detailed analysis of the core promoter was performed. Rapid amplification of cDNA ends (RACE) and RNase protection methods showed two

major transcriptional initiation sites. Luciferase reporter assays using 5' promoter deletion-reporter constructs containing up to 3 kb of 5' sequence were performed in hematopoietic and small-cell lung cancer cell lines which either did or did not express the endogenous c-kit gene. This analysis showed the region 83 to 124 bp upstream of the 5' transcription initiation site was crucial for maximal core promoter activity. Sequence analysis showed several potential Sp1 binding sites within this highly GC-rich region. Gel shift and DNase footprinting showed that Sp1 selectively bound to a single site within this region. Supershift studies using an anti-Sp1 antibody confirmed specific Sp1 binding. Site-directed mutagenesis of the -93/-84 Sp1 binding site reduced promoter-reporter activity to basal levels in c-kit-expressing cells. Cotransfection into Drosophila SL2 cells of a c-kit promoter-reporter construct with an Sp1 expression vector showed an Sp1 dose-dependent enhancement of expression that was markedly attenuated by mutation of the -93/-84 site. These results indicate that despite the fact that the human c-kit promoter contains multiple potential Sp1 sites, Sp1 binding is a selective process that is essential for core promoter activity.

Pfau W, Martin FL, Cole KJ, Venitt S, Phillips DH, Grover PL, Marquardt H. **Heterocyclic aromatic amines induce DNA strand breaks and cell transformation.** Carcinogenesis 1999;20(4):545-51. Heterocyclic aromatic amines (HAAs), formed during the cooking of foods, are known to induce tumours in rodent bioassays and may thus contribute to human cancer risk. We tested six HAAs in a morphological transformation assay and in three in vitro genotoxicity assays. The morphological transforming abilities of HAAs were tested, in the presence of rat-liver S9, in the C3H/M2 fibroblast cell line. Concentration levels of 50 microM 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (8-MeIQx), 100 microM 2-amino-3,4,8-trimethylimidazo-[4,5-f]quinoxaline (4,8-DiMeIQx), 50 microM 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 100 microM 2-amino-9H-pyrido[2,3-b]indole (AalphaC), 100 microM 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAalphaC) and 15 microM 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induced maximum transformation potencies of 5.5, 6.6, 6.3, 5.2, 7.3 and 9.2 transformed foci per 10(4) surviving cells, respectively. Bacterial mutagenic activity was determined in the presence of rat-liver S9 using the Salmonella typhimurium reverse-mutation assay employing strain YG1019. Mutagenic potencies of 3800 revertants (revs)/ng with 8-MeIQx, 2900 revs/ng with 4,8-DiMeIQx, 3480 revs/ng with IQ, 1.6 revs/ng with AalphaC, 2.9 revs/ng with MeAalphaC and 5 revs/ng with PhIP were observed. Clastogenic activity in vitro was analysed by the micronucleus assay in metabolically competent MCL-5 cells. Dose-dependent induction of micronuclei was observed for all HAAs tested with 1-5.4% of cells containing micronuclei at 10 ng/ml. Micronucleus induction was in the order 4,8-DiMeIQx > 8-MeIQx > IQ > MeAalphaC > PhIP > AalphaC. DNA strand-breaking activity in MCL-5 cells was measured by the alkaline single cell-gel (comet) assay. The lowest effect doses for significant increases ($P < or = 0.0007$, Mann-Whitney test) in comet tail length (microm) were 45.5 microg/ml (200 microM) for PhIP, 90.9 microg/ml (410-510 microM) for 4,8-DiMeIQx, IQ, MeAalphaC and AalphaC, and 454.5 microg/ml (2130 microM) for 8-MeIQx. It is not yet clear which of these assays most accurately reflects the genotoxic potential to humans of compounds of this class of environmental carcinogens.

Pogozelski WK, Xapsos MA, Blakely WF. **Quantitative assessment of the contribution of clustered damage to DNA double-strand breaks induced by 60Co gamma rays and fission neutrons.** Radiat Res 1999;151(4):442-8.

The induction of DNA strand breaks by fission neutrons was studied in aqueous plasmid (pBR322) DNA under aerobic conditions for a wide range of hydroxyl radical (*OH) scavenger concentrations and was compared to the induction of strand breaks by 6OCo gamma rays. Strand breaks were measured using agarose gel electrophoresis coupled with sensitive 32P-based phosphor imaging. Yields are reported for DNA single-strand breaks (SSBs) and double-strand breaks formed linearly with dose (alphaDSBs). The fraction of alphaDSBs that were dependent on the multiply damaged site (MDS) or clustered damage mechanism was also calculated using a model. G values for SSBs and alphaDSBs declined with increasing *OH scavenging capacity. However, with increasing *OH scavenging capacities, the decrease in yields of strand breaks for fission neutrons was not as pronounced as for gamma rays. The percentage of alphaDSBs for gamma rays was dependent on *OH scavenging capacity, appearing negligible at low scavenging capacities but increasing at higher scavenging capacities. In contrast, fission neutrons induced high percentages of alphaDSBs that were approximately independent of *OH scavenging capacity. The levels of alphaDSBs formed by the MDS mechanism after exposure to fission neutrons are consistent with the expected distinctive features of high-LET energy deposition events and track structure. The results also confirm observations made by others that even for low-LET radiation, the MDS mechanism contributes significantly to DNA damage at cell-like scavenging conditions.

Ramirez MJ, Surralles J, Puerto S, Creus A, Marcos R. **Low persistence of radiation-induced centromere positive and negative micronuclei in cultured human cells.** Mutat Res 1999;440(2):163-9.
The micronucleus (MN) assay is widely used both in genetic toxicology and in the biomonitoring of human populations. Lymphocytes, cell lines, and bone marrow and epithelial cells are usually employed as target systems in such studies. However, little effort has been done to assess the persistence of MN in highly proliferative cells. To study the behaviour of MN containing whole chromosomes or acentric fragments, we have performed a time course experiment on the persistence of gamma-ray (3 Gy) induced MN in a human lymphoblastoid cell line. The frequency and content of MN were analyzed 1, 3, 7, 14, and 56 days after irradiation by pancentromeric fluorescence in situ hybridization (FISH). We observed a clear induction of both centromere positive and negative MN at completion of the first mitotic division. The frequency of both types of MN drastically declined to basal levels 7 days after irradiation with an identical kinetics. We therefore conclude that centromere positive and negative MN are highly unstable upon cell division, indicating that the MN assay could not be a good biomarker of DNA damage induced by acute treatments in highly proliferative cells. The implication of our findings in biomonitoring and in genotoxicity studies is discussed. Copyright 1999 Elsevier Science B. V.

Risom L, Knudsen LE. **Use of cryopreserved peripheral mononuclear blood cells in biomonitoring.** Mutat Res 1999;440(2):131-8.
This study was performed to investigate the effect of storing blood samples by freezing on selected biomarkers and possible implications for biomonitoring. Comparative measurements were performed in order to investigate the use of cryopreserved vs. freshly separated peripheral mononuclear blood cells (PMBC) obtained from donor blood. Measurements of DNA-repair, mutant frequency, and subcell content were included. Samples for large biomonitoring studies are usually taken from study groups within a short time period of days/weeks and storing of study material for later analysis can be

necessary. We measured the DNA repair activity as dimethylsulfate induced unscheduled DNA synthesis (UDS) in PMBC incubated with either autologous plasma or fetal bovine serum (FBS). Comparison of the hprt mutant frequency by the T cell cloning assay was made in parallel. Finally the content of B/T-lymphocytes and monocytes was measured in phytohemaglutinin (PHA)-stimulated cultures at different time intervals. The results showed a higher DNA repair activity in cryopreserved samples compared with fresh samples. We also found differences in mutant frequencies with higher values in fresh samples. A significant correlation of frequencies was seen when comparing fresh with cryopreserved samples. Furthermore we recommend fresh human plasma used in UDS incubation media. Copyright 1999 Elsevier Science B.V.

Rodriguez-Ariza A, Alhama J, Diaz-Mendez FM, Lopez-Barea J. **Content of 8-oxodG in chromosomal DNA of Sparus aurata fish as biomarker of oxidative stress and environmental pollution.** Mutat Res 1999;438(2):97-107.
The 8-oxodG content has been measured in chromosomal DNA of gilthead seabream (Sparus aurata) by HPLC-EC. Susceptibility of different tissues to oxidative DNA damage was studied by exposing fish to model pollutants. Cu(II), paraquat (PQ) and malathion failed to promote DNA oxidation in liver, while dieldrin significantly increased the 8-oxodG content in this organ, but not in gills or blood. After PQ exposure, fish liver showed high levels of glucose-6-P dehydrogenase (G-6PDH) and GSSG reductase activities. The increased antioxidant status and the lack of a specific transport system could explain the lack of susceptibility of liver to DNA oxidative damage induced by PQ. Increased levels of 8-oxodG were detected in the gills of PQ-exposed fish after 8 and 24 h. In contrast, after 48 h exposed fish contained lower 8-oxodG levels than controls. The existence of a PQ transport system in this O2-rich organ and the lack of a significant increase in antioxidant defenses would explain the sensitivity of gills to DNA damage promoted by PQ. Elimination of this soluble chemical and the putative induction of DNA-repair enzymes specific for oxidative damages could explain the drop of 8-oxodG levels at longer times. Fish exposed to moderate levels of urban and industrial pollution showed significantly high 8-oxodG content in hepatic DNA. We conclude that 8-oxodG determination in chromosomal DNA by HPLC-EC is a potentially useful biomarker of environmental pollution, although its response is still somewhat lower than that of other well-established biomarkers of oxidative stress. Copyright 1999 Elsevier Science B.V.

Rojas E, Lopez MC, Valverde M. **Single cell gel electrophoresis assay: methodology and applications.** J Chromatogr B Biomed Sci Appl 1999;722(1-2):225-54.
The single cell gel electrophoresis or Comet assay is a sensitive, reliable, and rapid method for DNA double- and single-strand breaks, alkali-labile sites and delayed repair site detection, in eukaryotic individual cells. Given its overall characteristics, this method has been widely used over the past few years in several different areas. In this paper we review the studies published to date about the principles, the basic methodology with currently used variations. We also explore the applications of this assay in: genotoxicology, clinical area, DNA repair studies, environmental biomonitoring and human monitoring.

Ruiz-Laguna J, Pueyo C. **Hydrogen peroxide and coffee induce G:C-T:A transversions in the lacI gene of catalase-defective Escherichia coli.** Mutagenesis 1999;14(1):95-102.

BIOSIS COPYRIGHT: BIOL ABS. The mutagenicity of hydrogen peroxide (H202) was compared with that of coffee, a complex mixture which generates H2O2. An Escherichia coli strain defective in catalase activity (katG katE double mutant) and carrying a single copy mucAB (pRW144) plasmid was constructed to enhance the mutagenic response to oxidants. The ability of the mucAB genes to influence the type, frequency and distribution of H2O2-induced mutations was also investigated in isogenic bacteria lacking pRW144. Induced mutational spectra were characterized and compared with that of spontaneous mutagenesis. A total of 444 independent, forward mutations affecting the first 210 hp of the lacI gene were identified by DNA sequence analysis. The spontaneous mutation spectrum showed no bias (P = 0.52) for substitutions at G:C base pairs. In contrast, in the H2O2-induced spectrum substitutions occurred preferentially at G:C base pairs (P < 0.0001) with a preponderance of G:C-T:A transversions (43.4% of H2O2-induced mutants versus 17.3% of spontaneous mutants). These data support the view that 7,8-dihydro-8-oxoguanine is the main premutagenic lesion induced by H2O2 and that catalase-defective bacteria have elevated levels of 8-oxoguanine in chromosome DNA after H2O2 exposure. Coffee produced a similar distribution of mutational events as H2O2 (P > 0.05), suggesting that this compound may be the main cause of the coffee-induced mutagenesis. The presence of plasmid pRW144 did not affect the frequency of H2O2-induced G:C-T:A transversions, but caused an increase in A:T-T:A transversions and a decrease in -1 base frameshifts. Although the frequencies of G:C-T:A transversions were similar in all three induced spectra (H2O2 and coffee : pRW144), differences were observed in location of mutations throughout the target gene.

Sanford KK, Parshad R. **The contribution of deficient DNA repair to chromosomal radiosensitivity of CHO cells after G2 irradiation.** Cancer Genet Cytogenet 1999;108(1):38-41.
We compared cytogenetic responses of the parental Chinese hamster ovary (CHO) cell line and its DNA repair-deficient strains to irradiation during the G2 phase. Chromatid breaks were quantified in cells entering metaphase in the presence or absence of cytosine arabinoside (ara-C) 0.5-1.5 hours after exposure to x-rays or UV-C. Addition of ara-C, an inhibitor of DNA repair replication, significantly increased chromatid break frequency (CBF) in the parental line, but not in the strains deficient in nucleotide excision repair (NER). This increase (ara-C effect) was comparable to that in repair-proficient normal human lymphocytes. We conclude that CBF in cells entering metaphase in the presence of ara-C 0.5-1.5 hours after DNA damage represents a functional in vitro assay for evaluating the DNA repair capacity of mammalian cells in culture.

Sasaki YF, Fujikawa K, Ishida K, Kawamura N, Nishikawa Y, Ohta S, Satoh M, Madarame H, Ueno S, Susa N, et al. **The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and U.S. NTP.** Mutat Res 1999;440(1):1-18.
The genotoxicity of 30 aromatic amines selected from IARC (International Agency for Research on Cancer) groups 1, 2A, 2B and 3 and from the U.S. NTP (National Toxicology Program) carcinogenicity database were evaluated using the alkaline single cell gel electrophoresis (SCG) (Comet) assay in mouse organs. We treated groups of four mice once orally at the maximum tolerated dose (MTD) and sampled stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow 3, 8 and 24 h after treatment. For the 20 aromatic amines that are rodent carcinogens, the assay was positive in at least one organ, suggesting a high predictive ability for the assay. For most of the SCG-positive aromatic amines, the organs exhibiting increased levels of DNA damage were not necessarily the target organs for

carcinogenicity. It was rare, in contrast, for the target organs not to show DNA damage. Organ-specific genotoxicity, therefore, is necessary but not sufficient for the prediction of organ-specific carcinogenicity. For the 10 non-carcinogenic aromatic amines (eight were Ames test-positive and two were Ames test-negative), the assay was negative in all organs studied. In the safety evaluation of chemicals, it is important to demonstrate that Ames test-positive agents are not genotoxic in vivo. Chemical carcinogens can be classified as genotoxic (Ames test-positive) and putative non-genotoxic (Ames test-negative) carcinogens. The alkaline SCG assay, which detects DNA lesions, is not suitable for identifying non-genotoxic carcinogens. The present SCG study revealed a high positive response ratio for rodent genotoxic carcinogens and a high negative response ratio for rodent genotoxic non-carcinogens. These results suggest that the alkaline SCG assay can be usefully used to evaluate the in vivo genotoxicity of chemicals in multiple organs, providing for a good assessment of potential carcinogenicity. Copyright 1999 Elsevier Science B.V.

Schaumloffel N, Gebel T. **Heterogeneity of the DNA damage provoked by antimony and arsenic.** Mutagenesis 1998;13(3):281-6.
Data on the mechanism of antimony genotoxicity is scarce. Arsenic and antimony are proposed to share some toxicological features. Thus comparative and combined experiments with As(III) and Sb(III) were performed to gain a deeper knowledge of the mechanism of antimony genotoxicity. Trivalent arsenic proved to be five times more cytotoxic and one order of magnitude more potent in induction of micronuclei in human lymphocytes in vitro than was antimony. Significantly increased micronucleus frequencies were achieved with As(III) at a dose of 0.5 microM and with Sb(III) at a dose of 5 microM. Neither the number of micronuclei induced by As(III) nor by Sb(III) could be suppressed by co-incubation with superoxide dismutase or catalase. This suggests that induction of oxidative stress may not be a crucial step in the mechanism of DNA damage induction by arsenic and antimony. The combined genotoxicity in micronucleus test co-incubation experiments with arsenic and antimony seemed best described by simple additivity. In the single cell gel test with human lymphocytes a significant induction of DNA damage was observed with 0.01 microM As(III) and 5 microM Sb(III). In contrast to Sb(III), As(III) proved to be a very potent inducer of DNA-protein crosslinks. It may be that Sb(III) as well as As(III) causes DNA damage by inhibition of enzymes involved in DNA repair. Further investigations will have to identify the relevant sites of action.

Schildcrout JS, Margolin BH, Zeiger E. **Predicting rodent carcinogenicity using potency measures of the in vitro sister chromatid exchange and chromosome aberration assays.** Environ Mol Mutagen 1999;33(1):59-64.

Schrader TJ. **Comparison of HepG2 feeder cells generated by exposure to gamma-rays, X-rays, UV-C light or mitomycin C for ability to activate 7,12-dimethylbenz[a]anthracene in a cell-mediated Chinese hamster V79/HGPRT mutation assay.** Mutat Res 1999;423(1-2):137-48.
The cell-mediated Chinese hamster V79/HGPRT mutagenicity assay is an established in vitro testing method. Although gamma-irradiated human HepG2 hepatoma cells have been used recently for chemical activation, an alternative is now needed due to scheduled retirement of the available gamma-source. X-irradiation, 254 nm UV-C light and mitomycin C were examined as possible HepG2 mitotic inhibitors, and treated cells compared for activation of 7, 12-dimethylbenz[a]anthracene (DMBA). In

colony-forming assays, V79 and HepG2 cells differed in sensitivity to DMBA, with V79 survival declining sharply between 1-2.5 microM (LD50=1.75 microM) while HepG2 survival decreased gradually, beginning at 0.01 microM DMBA (LD50=0.045 microM). When HepG2 feeder cells generated by each method were included in V79/HGPRT mutation assays, activation of 1 microM DMBA was found to vary according to the mitotic inhibitor used, with mutation frequencies decreasing in the order 4000 rads gamma-rays>25 microg/ml mitomycin C>4000 rads X-rays>25 J/m2 UV-C light. Only assays containing gamma-irradiated HepG2 cells generated an increase (2-3-fold) in mutation frequency when DMBA exposure was extended from 24 to 48 h. The effect of HepG2 preincubation with either Aroclor 1254 or DMBA on feeder cell activation of DMBA was also assessed using concentrations of Aroclor 1254 (10 microg/ml) or DMBA (1.0 microM) which were found to produce optimum induction of ethoxyresorufin-O-deethylase (EROD) activity (3.1-fold and 2-fold increases, respectively). Compared to results obtained with uninduced HepG2 cells, assays incorporating HepG2 cells activated by either Aroclor 1254 or DMBA produced slightly increased V79/HGPRT mutation frequencies after 24 h of exposure to mutagen; however, a 48 h incubation with mutagen in the presence of HepG2 preincubated with either Aroclor 1254 or DMBA resulted in higher mutation frequencies regardless of the mitotic inhibitor treatment. EROD activity was also induced 1.4-fold following exposure of HepG2 cells to mitomycin C alone. Although gamma-irradiation remains the treatment of choice for producing metabolically active HepG2 feeder cells, comparison of the alternatives tested suggests that mitomycin C would be a convenient and suitable replacement.

Seel K, Walber U, Herbold B, Kopp R. **Chemical behaviour of seven aromatic diisocyanates (toluenediisocyanates and diphenylmethanediisocyanates) under in vitro conditions in relationship to their results in the Salmonella/microsome test.** Mutat Res 1999;438(2):109-23.
There are conflicting results on the mutagenicity of toluenediisocyanate (TDI) and diphenylmethanediisocyanate (MDI). It was found that the organic solvent chosen to dissolve the compounds dictates the outcome of the bacterial tests. The Salmonella/microsome tests showed uniformly mutagenic effects for all the compounds that were predissolved in DMSO. Due to the instability of aromatic diisocyanates in DMSO this solvent was replaced by ethyleneglycoldimethylether (EGDE). TDI and MDI endured the dissolving and were therefore still available for the subsequent bacterial tests. Furthermore, no aromatic diamines (TDA or MDA) could be detected in EGDE prior to the start of the assays. The Salmonella/microsome tests, however, revealed unexpected differences between TDI and MDI. As previously published the four types of MDI showed negative results, whereas the data presented in this paper demonstrated mutagenic effects of all three types of TDI if EGDE is the solvent. To gain deeper insight into the chemical changes that occurred during the Salmonella/ microsome test, the possible reactions were modelled in the laboratory by mixing predissolved diisocyanates with a defined surplus of water and monitoring the progress of the chemical reactions by analytical methods. Additionally, the quality of the model was checked by exposing solutions of 2,6-TDI and 4,4'-MDI to the real biological test environment. In both cases, the reaction patterns of TDI were different to those of MDI. Within 1 min, which is the maximum time needed to mix the predissolved compounds with water before they are poured onto the agar plate, the TDI content was reduced in favour of different ureas and TDA. In addition water was replaced by the complete set of test ingredients. While the TDA content remained more or less constant, the amount of residual TDI was reduced considerably.

Reactions of MDI were markedly slower than those of TDI. More than 90% of the predissolved MDI remained intact when it was mixed with water. The biological test ingredients accelerated the reduction of the MDI content. Within 45 s, more than two thirds of the MDI disappeared. Evidently, the chemical reactions continue during incubation. It is assumed that the contrasting results of TDI and MDI in the Salmonella/microsome test are due to the different reaction patterns-and reaction products-of the predissolved diisocyanates created under the specific conditions of the test. These findings indicate that the chemical interactions between reactive test compounds and solvents or test media need to be considered in the interpretation of the relevance of test results. Copyright 1999 Elsevier Science B.V.

Singh J, Mclean JA, Pritchard DE, Montaser A, Patierno SR. **Sensitive quantitation of chromium-DNA adducts by inductively coupled plasma mass spectrometry with a direct injection high-efficiency nebulizer.** Toxicol Sci 1998;46(2):260-5.
A novel method is described for the sensitive detection of chromium-DNA adducts. Chromium-DNA adducts were determined in 1 microgram of DNA from normal human lung fibroblasts exposed to sodium chromate using microscale flow injection analysis with a direct injection high-efficiency nebulizer and inductively coupled plasma mass spectrometry detection. The frequency of Cr-DNA adducts increased in a dose-dependent sigmoidal manner, indicating saturation and toxicity. The low detection limits (on the order of parts per trillion) allows the detection of as few as two Cr adducts per 10,000 bases, which, coupled with the small DNA sample requirement, makes this technique suitable for measuring metal-DNA adducts as biomarkers of exposure to toxic and carcinogenic metals such as Cr, in cultured cells, animals, and humans.

Stavreva DA, Ptacek O, Plewa MJ, Gichner T. **Single cell gel electrophoresis analysis of genomic damage induced by ethyl methanesulfonate in cultured tobacco cells.** Mutat Res 1998;422(2):323-30.
A procedure for employing cultured tobacco cells (line TX1) in the SCGE assay was developed. The effect on DNA migration was studied in control and EMS-treated cells at different stages in their growth curve. The experimental parameters of treatment time and the unwinding time were analyzed in TX1 cells. With EMS in a concentration range from 0 to 30 mM the average median (+/-S.E.) tail moments ranged from 2.71+/-0.24 microm for the negative controls and increased in a direct concentration dependent manner to 57.89+/-4.13 for cells treated with 30 mM EMS. Nuclei isolated from TX1 cells and treated with EMS had a similar sensitivity as TX1 cells after EMS treatment. The plant cells express similar concentration-response curves for EMS as reported with mammalian (CHO) cells. This plant cell SCGE assay may prove to be a useful tool for the study of agricultural chemicals in specific plant cell types, to compare the response of mutagens in plant and animal cells and for basic research in genetic toxicology and DNA repair in plants. Copyright 1998 Elsevier Science B.V.

Sun JT, Armstrong MJ, Galloway SM. **Rapid method for improving slide quality in the bone marrow micronucleus assay; an adapted cellulose column procedure.** Mutat Res 1999;439(1):121-6.
Micronuclei are routinely scored in anucleate erythrocytes in bone marrow smears stained with acridine orange. Intense fluorescence from the many nucleated cells in the preparations can interfere with micronucleus detection and cause fatigue in the reader. A method for removing nucleated cells by filtering bone marrow through cellulose packed in syringes was developed by Romagna some ten years

ago, but has not been used routinely because of the excessive time needed to prepare columns. We have modified the method very simply by filling chromatography columns by pipet with a cellulose suspension. We show here that column filtration of bone marrow does not affect the numbers of micronucleated polychromatic erythrocytes (MN-PCEs) scored from mice treated with the chromosome breaking agents mitomycin C and cyclophosphamide, or the aneuploidy-inducing spindle poisons, colchicine and vinblastine. The extra preparation time is only about half an hour for a full scale micronucleus assay, and results in better slides and faster scoring. Copyright 1999 Elsevier Science B.V.

Tessitore A, Pastore L, Rispoli A, Cilenti L, Toniato E, Flati V, Farina AR, Frati L, Gulino A, Martinotti S. **Two gamma-interferon-activation sites (GAS) on the promoter of the human intercellular adhesion molecule (ICAM-1) gene are required for induction of transcription by IFN-gamma.** Eur J Biochem 1998;258(3):968-75.
We describe the molecular features of the interferon (IFN)-gamma-mediated transcription of the human intercellular adhesion molecule (ICAM-1) gene. We identified putative IFN-gamma-activated sites (GAS) distributed throughout a large segment of the ICAM-1 promoter (4.0 kb region). Using computer-assisted search, these sequences were similar to potential IFN-gamma responsive elements that have a core sequence 5'-TTNCNNNAA-3'. In this report we show that in the ICAM-1 promoter a GAS site is located at -115 from the translation initiation site, and binds with strong affinity to IFN-gamma-activated Signal Transducers and Activators of Transcription (STAT1) homodimers. The same sequence is responsible for the IFN-gamma-mediated transcription of the ICAM-1 gene. Moreover, we present evidence that a more distal GAS element that maps at -2787 from the translation initiation site, binds IFN-gamma-activated STAT1 dimers with lower affinity. Multimeric copies of such GAS sequence inserted into a tkCAT minimal promoter can drive transcription, demonstrating that the -2787 bp GAS element has an independent functional activity upon binding of IFN-gamma-activated STAT1 proteins as documented by in vitro binding assays. Furthermore, using recombinant ICAM-CAT mutants, we show that, in vivo, the -2787 GAS, but not a mutagenized -2787 GAS site, when coupled to the more proximal -115 GAS element, has an additive effect in enhancing the IFN-gamma-mediated transcription of ICAM-1 promoter. Nevertheless, using a recombinant construct bearing the wild type -2787 GAS element and a mutagenized -115 GAS element, we could not detect any transcription after transfection of U937 recipient cells, suggesting that the -2787 bp GAS element is not sufficient as such for gene activation, but can cooperate with its cognate proximal sequence to give full function to the ICAM-1 promoter during the IFN-gamma response. Taken together these data provide evidence that two GAS sites are required for the full potential activity in the mechanism of ICAM-1 gene activation by IFN-gamma.

Vock EH, Vamvakas S, Gahlmann R, Lutz WK. **Investigation of the induction of DNA double-strand breaks by methylenediphenyl-4-4'-diisocyanate in cultured human lung epithelial cells.** Toxicol Sci 1998;46(1):83-9.
The question was addressed whether methylenediphenyl-4,4'-diisocyanate (MDI), a bifunctional electrophile, can induce DNA double-strand breaks (DSB) by repair of interstrand DNA crosslinks or whether DSB are the result of cell death. Cultured human lung epithelial cells (A549) were treated with MDI, methylene-4,4'-dianiline (MDA; a potential hydrolysis product of MDI), the nitrogen mustard melphalan, and the detergent Triton X-100. All chemicals were dissolved in ethylene glycol dimethyl

ether which was added to a cell monolayer covered with phosphate-buffered saline. After 2 h, the treatment solution was exchanged against medium, and 8, 24, and 72 h after treatment initiation, the induction of DNA double-strand breaks was assessed by pulsed-field gel electrophoresis. At the same time, the viability was determined with the MTT test (intracellular reduction of the tetrazolium dye MTT). At the 8-h time point, 1 and 10 microM melphalan induced DSB without concomitant effect on cell viability. With all other chemicals, the dose-response curves for DNA fragmentation and viability were mirror images. Approximate 50% lethal concentrations were 200, 3000, and 100 microM for MDI, MDA, and Triton X-100, respectively. For these chemicals, the observed DSB were the consequence of extragenomic damage in the course of cell death rather than of an interaction with DNA. The mechanistic difference of melphalan was supported by analysis of nuclear morphology. Apoptotic bodies were observed only after melphalan treatment, whereas MDI and Triton X-100 produced only irregular clumping of chromatin (72-h time point). DNA fragment length analysis showed a time-independent pattern, with sizes between 1 and 4 Mbp for melphalan, while MDI, and Triton X-100 induced smaller DNA fragments in a time-dependent manner. It is concluded that DSB observed in cells treated with MDI are unlikely the result of DNA crosslink formation.

Wagner ED, Rayburn AL, Anderson D, Plewa MJ. **Analysis of mutagens with single cell gel electrophoresis, flow cytometry, and forward mutation assays in an isolated clone of Chinese hamster ovary cells.** Environ Mol Mutagen 1998;32(4):360-8.
We investigated the induction of DNA strand breaks in the single cell gel electrophoresis (SCGE or comet) assay with whole cell clastogenicity measured with flow cytometric analysis in cells from an isolated clone of the Chinese hamster ovary (CHO) AS52 cell line. Under identical treatment conditions the responses were compared with forward mutation at gpt using 2-acetoxyacetylaminofluorene (2AAAF), ultraviolet radiation (UV) and ethyl methanesulfonate (EMS). Cytotoxicity for each agent was evaluated in the SCGE and forward mutation assays. Forward mutation was 4-10-fold more sensitive than DNA strand breaks detected in the SCGE assay. For 2AAAF and EMS, the kinetics of the induction of genetic damage were similar for the three assays, although there were differences in sensitivity. With UV, the induction kinetics of gpt mutation differed from that expressed by SCGE and flow cytometric analysis. With the chemical mutagens 2AAAF and EMS, there was a high correlation between the SCGE assay and forward mutation and also between the SCGE assay and flow cytometry. There was no significant correlation between flow cytometry and forward mutation. With UV, only the SCGE assay and flow cytometry were correlated. Agent-specific variations in the intragenomic distribution of DNA damage for each mutagen was measured in the SCGE assay.

Zarani F, Papazafiri P, Kappas A. **Induction of micronuclei in human lymphocytes by organic solvents in vitro.** J Environ Pathol Toxicol Oncol 1999;18(1):21-8.
Our work is focused on identifying micronuclei (MN) induced in whole blood lymphocyte cultures after treatment with the organic solvents toluene, benzene, and acetone. We used the micronucleus test as a cytogenetic biomarker for genotoxicity and treated whole blood cultures with different concentrations of these solvents (0.1 to 5 mM) and mixtures of them (toluene plus acetone, toluene plus benzene). Our results did not show a significant increase in the number of micronuclei in binucleated lymphocytes after 48 hr of in vitro treatment. The addition of an external metabolic factor (10% S9 mix for 2 hr) in blood cultures treated with the organic solvents or their mixtures did not cause induction of MN. These results

indicate the lack of genotoxic activity of toluene, benzene, and acetone in vitro.

Zeiger E. **Identification of rodent carcinogens and noncarcinogens using genetic toxicity tests: premises, promises, and performance.** Regul Toxicol Pharmacol 1998;28(2):85-95.
The basic premises that guide genetic toxicity testing for identifying carcinogens and to support administrative and regulatory decisions are: the Salmonella mutagenicity test is a necessary component of testing schemes; a chromosome aberration test is needed in addition to a gene mutation test; a mammalian cell mutagenicity test is needed in addition to the Salmonella test; in vivo tests are needed to confirm the results of in vitro tests; and test batteries are more predictive than the individual tests of the battery. Results from the Salmonella mutagenicity, in vitro chromosome aberration, mutations in mouse lymphoma cells, rodent bone marrow micronucleus, and rodent carcinogenicity tests, performed by the U.S. National Toxicology Program, were used to evaluate these premises. A positive Salmonella test was most predictive of carcinogenicity. However, the data do not support using the other tests in addition to Salmonella for predicting carcinogenicity. The genetic toxicity tests did not complement each other, and batteries or combinations of the tests were no more predictive of carcinogenicity than Salmonella alone. If a chemical is mutagenic in Salmonella it should be considered a potential rodent carcinogen, unless ancillary information suggests otherwise. Positive responses in the other in vitro or in vivo tests do not increase the probability that the chemical is a carcinogen, and negative responses in the other tests do not diminish the implications of the positive Salmonella response.

HEPATIC AND RENAL TOXICITY

Hinton DE, Couch JA. **Architectural pattern, tissue and cellular morphology in livers of fishes: relationship to experimentally-induced neoplastic responses.** Exs 1998;86:141-64.
The teleost liver is one of the most sensitive organs to show alteration in biochemistry, physiology and structure following exposure to various types of environmental pollutants. Despite the importance of this organ to environmental toxicology and to ecotoxicology where biomarkers of exposure and of deleterious effect are found, the architectural pattern is not well known. This chapter reviews an architectural plan for teleost liver and compares that to the often cited mammalian pattern. Hepatic tubules composed principally of hepatocytes and biliary epithelial cells are in close proximity to lacunae which are of mesodermal origin. As is described, the tubule and lacunae concepts provide a means to better interpret morphologic alterations following exposure. These concepts are used to illustrate features of the chronic toxicity following exposure to proven carcinogens.

Ohno Y, Miyajima A, Sunouchi M. **Alternative methods for mechanistic studies in toxicology. Screening of hepatotoxicity of pesticides using freshly isolated and primary cultured hepatocytes and non-liver-derived cells, SIRC cells.** Toxicol Lett 1998;102-103:569-73.
We constructed a screening battery for the evaluation of hepatotoxicity using freshly isolated and primary cultured rat hepatocytes (abbreviated to FIH and PCH, respectively) and rabbit eye derived cell line, SIRC cells. Effects on cell viability and drug metabolizing enzyme activities were examined by several pesticides and compared to those of in vivo. Among the pesticides studied, prometryn and ametryn showed cytotoxicity on PCH at lower concentration than on SIRC cells. Cytotoxicities of these chemicals on FIH were inhibited by metyrapone. They also increased rat serum AST in vivo. On the

other hand, cytotoxicities of IBP, erusan, alanicarb, benfuracarb, and swep in PCH were observed at similar concentration to those in SIRC cells. Linuron, nitrofen, and chlomethoxifen increased ethoxycoumarin O-deethylation activities by almost similar concentration to those of benzo[a]pyrene. Linuron also induced ethoxycoumarin O-deethylation activity in vivo. These findings indicated that a battery of in vitro tests consisting of FIH, PCH and SIRC cells was useful to screen the hepatotoxicity of pesticides.

Rakba N, Melhaoui A, Loyer P, Guy Delcros J, Morel I, Lescoat G. **Bgugaine, a pyrrolidine alkaloid from Arisarum vulgare, is a strong hepatotoxin in rat and human liver cell cultures.** Toxicol Lett 1999;104(3):239-48.
Toxicity of bgugaine, a pyrrolidine alkaloid extracted from the tubers of Arisarum vulgare, was studied in three different liver cell culture models: (1) the rat hepatocyte primary culture; (2) a liver epithelial cell line; and (3) the human hepatoblastoma cell line HepG2. Cytotoxicity was evaluated by LDH release, MTT reduction and MDA production. DNA fragmentation was analysed by flow cytometry or DNA gel-electrophoresis. In hepatocyte and epithelial cell cultures, drug toxicity appeared at 30 microM and was evaluated by an increase in LDH release, a decrease in MTT reduction and a higher level of MDA production. Bgugaine concentrations lower than 30 microM did not induce changes in these parameters. In HepG2 cells, bgugaine treatment also induced LDH release at concentrations of 40 and 50 microM. DNA fragmentation, analysed in the HepG2 cell line by flow cytometry, was observed in cultures exposed to 50 microM bgugaine. However, using DNA gel-electrophoresis, we demonstrated that lower bgugaine concentrations (10, 20 and 30 microM) also induced DNA damage. Our results show that: (1) bgugaine induces an important hepatotoxicity; (2) bgugaine toxicity is not mediated by a metabolic derivative; and (3) bgugaine induces a significant DNA damage. Therefore, our data suggest that the alkaloid bgugaine contained in Arisarum vulgarae may be involved in the toxicologic symptoms observed after consumption of this plant tubers by humans and animals.

Shimizu I, Mizobuchi Y, Yasuda M, Shiba M, Ma YR, Horie T, Liu F, Ito S. **Inhibitory effect of oestradiol on activation of rat hepatic stellate cells in vivo and in vitro.** Gut 1999;44(1):127-36.
BACKGROUND: Hepatic stellate cells play a key role in the pathogenesis of hepatic fibrosis. AIMS: To examine the inhibitory effect of oestradiol on stellate cell activation. METHODS: In vivo, hepatic fibrosis was induced in rats by dimethylnitrosamine or pig serum. In vitro, rat stellate cells were activated by contact with plastic dishes resulting in their transformation into myofibroblast-like cells. RESULTS: In the dimethylnitrosamine and pig serum models, treatment with oestradiol at gestation related doses resulted in a dose dependent suppression of hepatic fibrosis with restored content of hepatic retinyl palmitate, reduced collagen content, lower areas of stellate cells which express alpha smooth muscle actin (alpha-SMA) and desmin, and lower procollagen type I and III mRNA levels in the liver. In cultured stellate cells, oestradiol inhibited type I collagen production, alpha-SMA expression, and cell proliferation. These findings suggest that oestradiol is a potent inhibitor of stellate cell transformation. CONCLUSION: The antifibrogenic role of oestradiol in the liver may contribute to the sex associated differences in the progression from hepatic fibrosis to cirrhosis.

IMMUNOTOXICITY

Boluda L, Sastre J, Casanovas M, Fernandez-Caldas E. **Determination of Ole e 1 by enzyme immunoassay and scanning densitometry: validation by skin-prick testing.** J Immunol Methods 1999;223(1):17-26.
BIOSIS COPYRIGHT: BIOL ABS. Ole e 1 is an important allergen in Olea europaea pollen extracts. This study describes the development of two new methods that can be used to estimate the Ole e 1 content in olive tree pollen extracts. They are based on (1) an enzyme immunoassay that uses rabbit polyclonal, monospecific antibodies and purified Ole e 1, and (2) scanning densitometry of SDS-PAGE gels. Twelve extracts were evaluated by in vivo and in vitro methods. The in vivo biological potency was estimated by prick skin testing 17 allergic individuals; the in vitro allergenic potency by direct IgE and IgE inhibition assays. The enzyme immunoassay showed an operative range of 0.03-100 mug/ml and demonstrated to be specific for Ole e 1. The Ole e 1 content ranged from 1% to 5% of the total protein in the 12 extracts. The amount of Ole e 1, assessed by gel scanning densitometry significantly correlated with the Ole e 1 content obtained by the immunoassay ($r = 0.92$; $p < 0.001$). The Ole e 1 content showed a significant correlation with the total allergenic potency of the extracts, evaluated by direct IgE. specific IgE inhibition and skin-prick testing. These two methods can be used to determine the Ole e 1 content in olive pollen extracts. The content of Ole e 1 can vary from 1% to 5% of the total protein in the extracts.

Bratel J, Jontell M, Dahlgren U, Bergenholtz G. **Effects of root canal sealers on immunocompetent cells in vitro and in vivo.** Int Endod J 1998;31(3):178-88.
Over the years of testing biocompatibility of endodontic filling materials, little attention has been paid to the potential adverse influences on the function of the immune system. Therefore, the purpose of this study was to investigate the extent to which extractable components of some commonly used root canal sealing materials (ERCS) may interfere with immunocompetent cells in vitro. The potential of these materials to cause delayed-type hypersensitivity (DTH) was also addressed in a rat model system. Extractable components were drawn in cell culture medium from freshly mixed or set material of AH 26. Grossman's sealer, Endomethasone, and Apexit. In-vitro assays included either spleen cells or rat pulp tissue cells that were released following enzymatic digestion with collagenase. Purified T cells for the pulpal cell assay were obtained from rat mesenteric lymph nodes. The effect of ERCS on the proliferation of concanavalin A (con A) stimulated spleen cell was measured by 3H-thymidine incorporation. Pulpal accessory cell function was monitored by the capacity of pulpal cells, pretreated with components of ERCS, to provide signals to con A stimulated T cells. DTH was tested after subcutaneous implantation of root canal sealers (RCS) in rats and challenge by ear injection. Pretreatment of pulpal cells with low dilutions of eluates from extracted AH 26 and Endomethasone resulted in a strong reduction of the T cell proliferation rate. The effect was considerably reduced ($P < 0.01$) when extracts of the solid material were employed. Extracts of Grossmans' sealer and Apexit affected T cell proliferation only to a limited extent in the pulpal cell assay. In general, assays on spleen cells showed a similar profile, although increased cell division was induced by Grossman's sealer at high eluate dilutions and a concentration-dependent decrease of cell division at lower concentrations of this material. ERCS evoked both immunosuppression and, in some instances, immunostimulation, but they did not release DTH.

Cederbrant K, Stejskal V, Broman P, Lindkvist L, Sundell K. **In vitro lymphocyte proliferation in the**

diagnosis of allergy to phenoxymethylpenicillin. Allergy 1998;53(12):1155-61.
BACKGROUND: The aim of this study was to investigate in vitro lymphocyte proliferation in the diagnosis of allergy to phenoxymethylpenicillin (PcV), comparing chemically reactive PcV, added to cell cultures in unconjugated form, to a PcV-PLL (poly-L-lysine) conjugate as antigens. Side-chain specificity of lymphoproliferative responses was investigated with reactive benzylpenicillin (PcG) and bacampicillin. METHODS: Seventeen patients with a history of hypersensitivity reactions in connection with PcV treatment were studied by means of the lymphocyte transformation test (LTT), the radioallergosorbent test (RAST), skin tests (prick and intracutaneous), and oral challenge with PcV. LTT was also performed in 20 control subjects exposed to PcV therapeutically, and in eight subjects with occupational exposure to this penicillin. RESULTS: Nine patients had a positive in vivo test to PcV (five by oral challenge, three by intracutaneous test, and one by both tests), and six were challenge-negative. When reactive PcV was used as antigen in LTT, positive LTT responses were observed in five of the nine patients with a positive in vivo test, and two of them were also side-chain specific. Positive LTT responses with reactive PcV also correlated with a positive RAST in five of seven subjects. None of the six patients with a negative challenge test, and only one of the 28 controls showed a positive LTT result with reactive PcV. Thus, the specificity of LTT with reactive PcV was 96%. In contrast, when PLL-conjugated PcV served as antigen, four challenge-negative subjects and 11 controls were LTT-positive. CONCLUSIONS: The results of this study indicate that LTT with chemically reactive PcV could be useful as an in vitro complement in the diagnosis of PcV allergy and as a tool to reveal the side-chain specificity of peripheral blood lymphocytes. A positive LTT to PLL-conjugated PcV may be an indicator of immunization, but not necessarily allergy, to the penicilloyl structure.

Della Gaspera B, Pham-Dinh D, Roussel G, Nussbaum JL, Dautigny A. **Membrane topology of the myelin/oligodendrocyte glycoprotein.** Eur J Biochem 1998;258(2):478-84.
Myelin/oligodendrocyte glycoprotein (MOG), a specific component of the mammalian central nervous system, is located on the surface of the oligodendrocyte plasma membrane and the outermost lamellae of mature myelin; it is expressed during the latter steps of myelinogenesis. It has been shown that MOG may play a pathological role in autoimmune demyelinating diseases of the central nervous system, although its physiological function remains unknown. MOG is an integral membrane glycoprotein with an extracellular immunoglobulin-like domain and two hydrophobic segments which were predicted to be membrane-spanning on the basis of hydropathy analysis. As a first step in elucidation of MOG function, we have investigated its membrane topology, combining immunofluorescence studies on cultured oligodendrocytes and MOG-transfected Chinese hamster ovary cells with biochemical analyses, including in vitro translation, membrane insertion and protease-digestion assays. Our results indicate that the C-terminal tail of MOG is located into the cytoplasm, and that only the first hydrophobic region of MOG spans the membrane whereas the second hydrophobic region appears to be semi-embedded in the lipid bilayer, lying partially buried in the membrane with its N-terminal and C-terminal boundaries facing the cytoplasm.

Hariya T, Hatao M, Ichikawa H. **Development of a non-radioactive endpoint in a modified local lymph node assay.** Food Chem Toxicol 1999;37(1):87-93.
A murine local lymph node assay (LLNA) has been developed as an alternative to guinea pig models for contact sensitization testing. Although the LLNA appears to be a little less sensitive than the most

stringent of guinea pig assays, it provides a rapid, objective, quantitative and cost-effective method for screening strong contact sensitizers and has advantages with respect to animal welfare. However, a potential disadvantage is the need for the use of radioactive material. We have reported previously that an ex vivo assay based on similar principles to the original in vivo LLNA, but using a non-radioactive endopoint, was valid for the prediction of strong sensitizers. This ex vivo assay was not sensitive enough to allow prediction of moderately potent ones. In this study, we propose a new parameter, Corrected IL-2 Index (CII), for the prediction of moderate sensitizers. To obtain CII the IL-2 release in the supernatant of the cell culture is corrected for lymph node weight ratio and ratio of CD4-positive subset. We found that CII predicted the allergenicity of moderate sensitizers, including the ones recommended by the OECD in guideline 406, such as mercaptobenzothiazole and hexyl cinnamic aldehyde. The allergenicity of metal salts, such as potassium dichromate, ammonium tetrachloroplatinate and cobalt chloride, was also predicted by the CII. We conclude that the use of CII as an index significantly increases the sensitivity of the ex vivo method so that moderate sensitizers may also be detected.

Montelius J, Wahlkvist H, Boman A, Wahlberg JE. **Murine local lymph node assay for predictive testing of allergenicity: two irritants caused significant proliferation.** Acta Derm Venereol 1998;78 (6):433-7.
The murine local lymph node assay is a method for predictive testing of contact allergenicity, but its ability to discriminate between allergens and irritants has been questioned. To explain some of the conflicting results with irritants, the proliferation induced by methyl salicylate and nonanoic acid, both considered to be non-sensitisers, was further investigated. Both substances showed a dose--response relationship and clearly positive results when tested at higher concentrations (> or = 50%) and would thus be classified as potential sensitisers according to the present criteria for a positive assay result. In the case of methyl salicylate, the use of either dimethyl formamide or methyl ethyl ketone as vehicle did not significantly influence the results. The negative results obtained for methyl salicylate in some earlier reports were probably due to testing at too low concentrations. The proliferation induced by irritants such as methyl salicylate and nonanoic acid and inter alia sodium dodecyl sulfate, Triton X-100, oxalic acid, chloroform/methanol (2:1) must be better recognized and elucidated before the assay can be generally accepted as a predictive test method.

Peterson AL, Qureshi MA, Ferket PR, Fuller JC. **In vitro exposure with beta-hydroxy-beta-methylbutyrate enhances chicken macrophage growth and function.** Vet Immunol Immunopathol 1999;67(1):67-78.
BIOSIS COPYRIGHT: BIOL ABS. beta-Hydroxy-beta-methylbutyrate (HMB), a leucine catabolite, has been shown to decrease broiler mortality. One possible target of HMB action may be the cells of the immune system. Macrophages from a chicken macrophage cell line, MQ-NCSU, were exposed to 0, 10, 20, 40, 80, and 100 mug of HMB per 5 x 104 cells in a 96-well culture plate, After 24 h of exposure, macrophage proliferation was quantitated by an MTT bioassay. In duplicate experiments, HMB stimulated growth over control ($p < 0.05$) at a wide range of doses. Macrophages were exposed to 20 and 80mug of HMB and the culture supernatant fractions tested for the presence of nitrite. HMB exposure (20 mug) increased nitrite production by 44.1% over the controls (Experiment 1, $p < 0.035$). To determine the phagocytic potential of macrophages after HMB exposure, MQ-NCSU cell line and Sephadex-G50 -elicited abdominal macrophages were incubated with fluorescent latex beads (1:40,

macrophage to beads ratio) for 1 h and then analyzed by flow cytometry. When exposed to 40 mug HMB, the phagocytic potential of MQ-NCSU macrophages was significantly higher (31.7%) than that of the controls ($p < 0.0006$). Sephadex-elicited macrophages exhibited 14.4% increased phagocytosis over controls when treated with 80 Ag HMB ($p < 0.0016$). When MQ-NCSU macrophages were exposed to HMB, Fc-receptor expression was significantly elevated over the controls ($p < 0.0001$). These data demonstrate that HMB exposure induces proliferation of macrophages in culture as well as enhances macrophage effector functions, such as nitrite production and phagocytosis. The findings of these studies imply that HMB can be used as a possible dietary immunomodulator.

Riccio A, Andreassi C, Eboli ML. **Antiphospholipid antibodies bind to rat cerebellar granule cells: the role of N-methyl-D-aspartate receptors.** Neurosci Lett 1998;257(2):116-8.
IgGs from sera containing antiphospholipid antibodies (aPL), detected as antibodies to cardiolipin, or control sera were incubated with rat cerebellar granule cells in primary culture. Using a mitochondrial dehydrogenase activity assay (MTT test), aPL IgGs were shown to decrease MTT metabolism after 24 h incubation with the cells, and to cause non-toxic amounts of glutamate to become neurotoxic when added to the cells for 45 min. Acute and chronic aPL toxicity were prevented by MK-801. Sera containing aPL bound to intact cerebellar neurons, as revealed by an immunofluorescent technique. These results suggest that antiphospholipid antibodies interfere with excitatory pathways in glutamatergic cerebellar granule cells by a mechanism involving overactivation of the NMDA glutamate receptor.

Rocke TE, Smith SR, Nashold SW. **Preliminary evaluation of a simple in vitro test for the diagnosis of type C botulism in wild birds.** J Wildl Dis 1998;34(4):744-51.
An enzyme-linked immunosorbent assay (ELISA) was developed for the detection of type C botulinum toxin (Clostridium botulinum) in wild birds. This simple, antigen-capture ELISA utilizes polystyrene immunosticks as the solid substrate, chicken antitoxin (IgY) as the coating antibody, rabbit antitoxin as the primary antibody, and peroxidase-labeled goat-anti-rabbit as the secondary antibody. To evaluate the immunostick ELISA as a diagnostic test for avian botulism, known concentrations of toxin were added to heparinized blood collected from healthy birds and tested by both the ELISA and mouse bioassay. Also, blood samples from 236 bird carcasses submitted to the National Wildlife Health Center (NWHC) for cause of death determinations were tested by both procedures. Using < or = 0.5 ml as the test volume for both procedures, the ELISA was less sensitive, detecting 0.25 ng/ml of toxin compared to 0.12 ng/ml for the mouse bioassay. Using the same volume of test sample for diagnostic submissions (< or = 0.5 ml), the ELISA was positive for 60% of the 149 clinically-diagnosed cases of botulism, whereas the mouse bioassay was positive for 79%. However, we demonstrated that with larger sample volumes (> or = 1.0 ml), the sensitivity of the ELISA may be equivalent or better than the mouse test due to the concentrating effect of the ELISA procedure. These preliminary results suggest that when adequate sample volumes are available, the immunostick ELISA can replace the mouse test for the diagnosis of botulism in wild birds.

St. Louis DC, Woodcock JB, Fransozo G, Blair PJ, Carlson LM, Murillo M, Wells MR, Williams AJ, Smoot DS, Kaushal S, et al. **Evidence for distinct intracellular signaling pathways in CD34+ progenitor$_{7}$to dendritic cell differentiation from a human cell line model.** J Immunol 1999;162

(6):3237-48.
Intracellular signals that mediate differentiation of pluripotent hemopoietic progenitors to dendritic cells (DC) are largely undefined. We have previously shown that protein kinase C (PKC) activation (with phorbol ester (PMA) alone) specifically induces differentiation of primary human CD34+ hemopoietic progenitor cells (HPC) to mature DC. We now find that cytokine-driven (granulocyte-macrophage CSF and TNF-alpha) CD34+ HPC-->DC differentiation is preferentially blocked by inhibitors of PKC activation. To further identify intracellular signals and downstream events important in CD34+ HPC-->DC differentiation we have characterized a human leukemic cell line model of this process. The CD34+ myelomonocytic cell line KG1 differentiates into dendritic-like cells in response to granulocyte-macrophage CSF plus TNF-alpha, or PMA (with or without the calcium ionophore ionomycin, or TNF-alpha), with different stimuli mediating different aspects of the process. Phenotypic DC characteristics of KG1 dendritic-like cells include morphology (loosely adherent cells with long neurite processes), MHC I+/MHC IIbright/CD83+/CD86+/CD14- surface Ag expression, and RelB and DC-CK1 gene expression. Functional DC characteristics include fluid phase macromolecule uptake (FITC-dextran) and activation of resting T cells. Comparison of KG1 to the PMA-unresponsive subline KG1a reveals differences in expression of TNF receptors 1 and 2; PKC isoforms alpha, beta I, beta II, and mu; and RelB, suggesting that these components/pathways are important for DC differentiation. Together, these findings demonstrate that cytokine or phorbol ester stimulation of KG1 is a model of human CD34+ HPC to DC differentiation and suggest that specific intracellular signaling pathways mediate specific events in DC lineage commitment.

Van Loveren H, De Jong WH, Vandebriel RJ, Vos JG, Garssen J. **Risk assessment and immunotoxicology.** Toxicol Lett 1998;102-103:261-5.
In general toxicity testing, maximal acceptable concentrations are derived from no-observed adverse effect levels (NOAEL) in rodents. Risk assessment then considers safety factors for the interspecies difference, and intraspecies variability. This approach can be used for assessing maximal acceptable concentrations for chemicals inducing direct immunotoxicity, resulting in e.g. reduced resistance to infections. As for predictive testing of chemicals in terms of sensitization, laboratory animal data are mostly used for risk assessment as well. Generally, the assessment of risk for chemicals that induce contact sensitivity is limited to hazard identification, and risk management is restricted to labeling. An alternative type of evaluation of the risk of adverse effects due to exposure to immunotoxic chemicals may be the so called parallellogram approach. In this parallellogram there are four cornerstones, one of which is the health effect of exposure to a chemical, assessed as an endpoint (e.g. infection model) in experimental animals, and another the quantitative prediction of this endpoint in humans. The other cornerstones are assays of parameters that are relevant to the mechanism of the adverse effect in experimental animals and humans, and are used for species comparison. Species comparisons between the animal species used for hazard identification and humans are crucial for extrapolation of animal data to the human situation. This approach can be used to provide relevant information on the dose-response relationship in humans. In concert with information on actual exposure, such data can then be used for the characterization of risk for adverse health effects in humans. Such approaches have been used for chemicals that exert direct immunotoxic activity (bis(tri-n-butyltin)oxide (TBTO)), and may hold promise for the risk evaluation of chemicals that exert skin sensitizing properties.

Woolhiser MR, Hayes BB, Meade BJ. **A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals.** Toxicol Methods 1998;8(4):245-56.
BIOSIS COPYRIGHT: BIOL ABS. In an effort to establish a single, rapid screening procedure for the sensitization and irritancy potential of new chemicals, the parameters of a murine Local Lymph Node Assay and a mouse ear swelling irritancy assay were combined. To validate this assay, a range of chemical irritants and sensitizers were evaluated for their ability to elicit responses in B6C3F1 female mice. Chemicals were administered for four consecutive days to the dorsal and ventral surfaces of each ear An increase in ear thickness served to predict irritancy, while (3H)thymidine uptake by cervical draining lymph nodes suggested sensitization. All chemicals known to be potent chemical sensitizers (oxazolone, 2,4-dinitrofluorobenzene, toluene diisocyanate) produced a marked lymph node cell proliferation in this assay. Animals exposed to irritating agents (sodium lauryl sulfate, croton oil, tetradecane, nonanoic acid, and benzalkonium chloride) experienced a significant increase in ear swelling. In addition, these irritating agents elicited low-level lymphocyte proliferation. In cases where chemicals are considered to be both potent sensitizers and irritants (2,4-dinitrofluorobenzene, toluene diisocyanate, and benzalkonium chloride), robust increases in (3H)thymidine incorporation and ear swelling were demonstrated. Results were dose-responsive for all chemicals tested. The combined LLNA/ear swelling assay appears to be a reliable predictor of sensitization and irritancy potential, since it identified the activity of all eight chemicals tested. The advantages of this approach include a further reduction in the number of animals and time required to screen chemicals for both irritancy and/or sensitization potential. Although this assay does not have the capacity to discriminate between nonspecific lymph node proliferation and weak sensitizing ability of strong irritants, the information gained by the irritation component of the assay provides additional information when evaluating the significance of low-level lymphocyte proliferation in the LLNA. With further validation this assay could be useful as a common screening tool in the research and development of new chemical products.

Yamaguchi F, Takahashi Y, Furuhama K. **Application of in vitro methods using peripheral whole blood to selecting highly susceptible individuals among common squirrel monkeys (Saimiri sciureus) to bacterial lipopolysaccharides.** Food Chem Toxicol 1999;37(2-3):117-23.
The present study was designed to elucidate whether the individual susceptibility of common squirrel monkeys (Saimiri sciureus) to bacterial lipopolysaccharides (LPS) can be predicted by in vitro testing batteries performed in advance. Of the in vitro tests, the blastogenic response (n = 11) to LPS was determined by a micro-blood culture technique, and the production (n = 6) of cytokines such as tumour necrosis factor (TNF-alpha), interleukin-1 (IL-1beta) and interleukin-6 (IL-6) released into the culture medium was measured with an enzyme-linked immunosolvent assay (ELISA). In the blastogenic assay, four out of 11 animals showed an increase in the uptake of [3H]thymidine in a concentration-dependent manner (LPS-positive reaction), while seven remaining animals did not show any response to LPS (LPS-negative reaction). Among the cytokines employed, an elevation in TNF-alpha production was noted in three out of six animals employed without affecting IL-1beta and IL-6 productions. After the completion of in vitro examinations, LPS was administered subcutaneously at 0.3 mg/kg to these animals (n = 11) for 14 consecutive days. The six monkeys including either four animals showing a LPS-positive reaction or three animals having an increase in TNF-alpha production exhibited moribund conditions from days 3 to 12, and five remaining monkeys including five animals showing a LPS-negative reaction or three animals having a decrease in TNF-alpha production survived. The extrapolation rate from the in vitro

data to the in vivo results was over 80% (9/11) and 100% (6/6) in the blastogenic assay and TNF-alpha production, respectively. These results demonstrate that the in vitro methods can be available to selection of LPS-sensitive squirrel monkeys in advance.

NEUROTOXICITY

Andrews DL, Williams GS, Mahoney JC, West JR. **DNA fragmentation during exposure of rat cerebella to ethanol under hypoxia imposed in vitro.** J Neurobiol 1999;38(1):82-92.
BIOSIS COPYRIGHT: BIOL ABS. To gain a better understanding into the mechanisms of damage incurred by neurons in periods following heavy alcohol exposure during development, we used an in vitro system to monitor the effects of alcohol and hypoxia on cell survival and DNA integrity. Samples representing the first few hours of exposure to alcohol and hypoxia were compared to those resulting from hypoxia alone. Measurements were taken from cell counts using Trypan blue exclusion and TUNEL assays as well as digital scans of the ethidium bromide fluorescence of genomic DNA isolated from the treated tissue. We found that DNA degradation from hypoxia was accelerated by several hours in the presence of 100 mM ethanol. This result depended on age, with adult animals (>8 months) having a similar response to 4-day postnatal animals, while the effect on 10-day postnatal animals and those of intermediate age (45 days postnatal) was increasingly delayed. Different methods of inducing the processive degradation of DNA produced laddering typical of apoptosis, a biphasic degradative process, or patterns usually associated with necrosis.

Andrews T, Zhang P, Bhat NR. **TNFalpha potentiates IFNgamma-induced cell death in oligodendrocyte progenitors.** J Neurosci Res 1998;54(5):574-83.
Oligodendrocytes in multiple sclerosis brain may be under a direct attack by proinflammatory cytokines, particularly tumor necrosis factor-alpha (TNFalpha) and interferon-gamma (IFNgamma). In this study, we have examined the in vitro cytotoxic effects of the two cytokines, individually and in combination, on oligodendrocyte lineage cells using morphological criteria, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide reduction assay (MTT), terminal deoxynucleotide transferase-mediated dUTP nick end-labeling (TUNEL), and agarose-gel electrophoretic analysis of fragmented DNA. IFNgamma exerted a dose-dependent cytotoxic effect on cultured CG4 cells, an oligodendrocyte progenitor cell line, and in primary cultures of purified oligodendrocyte progenitors. TNFalpha, while by itself being only mildly toxic, greatly potentiated the cytotoxicity of IFNgamma. The cytokine effects were developmentally modified in that their cytotoxic and cooperative effects became less evident in more differentiated cells. A cell-permeable peptide inhibitor (i.e., z-VAD.fmk) of caspases partially suppressed apoptotic changes elicited by the cytokine combination in CG4 cells but not in primary oligodendrocytes. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of mRNA prepared from cytokine-treated cultures revealed an increased expression of the death receptor, Fas. The results suggest particular vulnerability of oligodendrocyte progenitors to a combination of TNFalpha and IFNgamma involving an activation of the cell death program.

Brown DR. **Prion protein-overexpressing cells show altered response to a neurotoxic prion protein peptide.** J Neurosci Res 1998;54(3):331-40.
A peptide fragment of the prion protein, PrP106-126 is toxic to neuronal cells in culture. This toxicity is

dependent on neuronal expression of the prion protein (PrPc) and also the presence of microglia. The role of expression of the PrPc in neurotoxicity of this peptide was investigated using mice that overexpress the prion protein. Cells derived from two different strains of PrPc-overexpressing mice were used (Tg20 and Tg35). PrP106-126 was more toxic to Tg35 cerebellar cells than wild-type or Tg20 cells. This increased toxicity required the presence of microglia. Analysis of microglia derived from wild-type and PrPc-overexpressing cells showed that Tg35 microglia were more easily activated than wild-type microglia, were more easily stimulated to proliferate by astrocytes, and had a higher level of PrPc expression. This may explain the increased PrP106-126 toxicity to Tg35 PrPc-overexpressing cerebellar cells. These results suggest that the toxicity of PrP106-126 may depend on the level of expression of PrPc by microglia as well as by neurones.

Bruinink A, Faller P, Sidler C, Bogumil R, Vasak M. **Growth inhibitory factor and zinc affect neural cell cultures in a tissue specific manner.** Chem Biol Interact 1998;115(3):167-74.
Deficiency of neuronal growth inhibitory factor (GIF) and abnormalities in zinc homeostasis have been suggested to play a role in the neuropathogenesis of Alzheimer's disease. We report here that embryonic chick cerebral cell cultures zinc and copper containing GIF in the presence of marmoset hippocampal extract reduces significantly and concentration dependently mitochondrial succinate dehydrogenase activity (MTT) and cell mass. In contrast, no indications could be found that GIF affected neural retina cell cultures. Our results suggest that the observed effects of GIF are not elicited by zinc.

Carlson RW, Bradbury SP, Drummond RA, Hammermeister DE. **Neurological effects on startle response and escape from predation by medaka exposed to organic chemicals.** Aquatic Toxicol 1998;43(1):51-68.
BIOSIS COPYRIGHT: BIOL ABS. Simultaneous electrophysiological and behavioral studies were performed on 21-32 day old juvenile medaka (Oryzias latipes) exposed at sublethal concentrations to organic chemicals representing various modes of action. Non-invasive recordings were made of the electrical impulses generated within giant neuronal Mauthner cells, associated interneurons and motoneurons, and axial musculature, all of which initiate the startle or 'escape' response in fish. Timing in ms between these electrical sequelae was measured for each fish before and after 24 and 48 h exposure to a chemical. Carbaryl and phenol affected Mauthner cell to motoneuron transmission while chlorpyrifos, carbaryl, phenol and 2,4-dinitrophenol (DNP) showed neuromuscular effects. The variety of neurological effects detected at various concentrations of chemicals tested here suggest that different mechanisms may be responsible. Also noted was the number of startle responses to number of stimuli ratio (R/S); this ratio was affected by most chemicals. Medaka generally appeared to be more susceptible to predation after exposure to chlorpyrifos, carbaryl. fenvalerate, endosulfan, phenol, 1-octanol and DNP. The effects threshold for many of the test compounds was found to be consistent for both the neurophysiological and behavioral endpoints. Consequently, electrophysiological responses of Mauthner cell-initiated startle responses provided a measure of neurological injury that is also directly correlated to a definitive and ecologically relevant behavioral endpoint.

Chen Q, Olney JW, Price MT, Romano C. **Biochemical and morphological analysis of non-NMDA receptor mediated excitotoxicity in chick embryo retina.** Vis Neurosci 1999;16(1):131-9.
Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate glutamatergic

neurotransmission, and when pathologically overstimulated induce excitotoxic neuronal death. Of the two families of iGluRs, the non-NMDA receptors have received less experimental attention than the NMDA receptors as mediators of neuronal death in in vitro systems. We have demonstrated that non-NMDA receptor activation is highly lethal for neurons of the chick embryo retina, and further characterize this phenomenon here. Treatment of isolated retinas with any of the non-NMDA receptor agonists glutamate, AMPA, or KA, in the presence of the NMDA receptor antagonist MK-801, led to pathomorphology and cell death. KA was the most effective toxin. All of KA-induced toxicity could be blocked by selective AMPA receptor blockers. The toxicity of both AMPA and glutamate could be greatly increased using cyclothiazide, which blocks AMPA receptor desensitization. These results indicate that KA is the most powerful toxin because it is a non-desensitizing agonist at the AMPA receptors. Glutamate exhibited a paradoxical ability to prevent KA-induced toxicity as measured by a biochemical assay of cell death. Also, histological studies indicated that glutamate selectively blocked KA-induced pathomorphological changes in bipolar cells. This protective effect of glutamate was not mimicked by AMPA, NMDA, or any of several metabotropic receptor agonists, indicating that it may be mediated by a receptor of undescribed pharmacology.

Cheung NS, Pascoe CJ, Giardina SF, John CA, Beart PM. **Micromolar L-glutamate induces extensive apoptosis in an apoptotic-necrotic continuum of insult-dependent, excitotoxic injury in cultured cortical neurones.** Neuropharmacology 1998;37(10-11):1419-29.
Excitotoxicity induced by L-glutamate (Glu), when examined in a pure neuronal cortical culture, involved widespread apoptosis at concentrations of 1-10 microM as part of a continuum of injury, which at its most servere was purely necrotic. Cells, maintained in chemically defined neurobasal/B27 medium, were exposed at d7 for 2 h to Glu (1-500 microM), and cellular injury was analysed 2 and 24 h after insult using morphology (phase-contrast microscopy), a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay, nuclear staining with 4,6-diamidino-2-phenylindole (DAPI), terminal transferase-mediated dUTP nick end-labelling (TUNEL) and DNA fragmentation by gel electrophoresis. Glu-mediated neurotoxicity was prevented by MK-801 (5 microM), whilst CNQX (20 microM) attenuated injury by 20%. Exposure to intensive insults (100 and 500 microM Glu) induced necrosis characterized by rapid cell swelling (< 2 h) and lack of chromatin condensation, confirmed by DAPI nuclear staining. In contrast, mild insults (< 20 microM Glu) failed to produce acute neuronal swelling at < 2 h, but 24 h after injury resulted in a large number of apoptotic nuclei as confirmed by TUNEL and electrophoretic evidence of DNA fragmentation, which was attenuated by cycloheximide (0.1 microg/ml). Our findings indicate for the first time that physiological concentrations of Glu produce neuronal injury across a continuum involving apoptosis (< 20 microM) and increasingly necrosis(> 20 microM), dependent on the severity of the initial insult.

Costa LG. **Biochemical and molecular neurotoxicology: relevance to biomarker development, neurotoxicity testing and risk assessment.** Toxicol Lett 1998;102-103:417-21.
Biochemical and molecular approaches are most useful to define potential mechanisms of neurotoxicity. Information on the mechanisms of action of neurotoxicants can play a key role in neurotoxicology by allowing, among others, the development of potential biomarkers of effect, the refinement of in vitro testing procedures, and the improvement of the risk assessment process. An important class of insecticides, the organophosphates, are discussed as an example of how knowledge of molecular

mechanisms is useful in various aspects of neurotoxicology. The utilization of such information in the area of biomarkers of exposure and effects, and of in vitro testing is presented. Additionally, mechanistic issues related to genetic polymorphisms and risk assessment are discussed.

Gerlai R. **A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study.** Behav Brain Res 1998;95(1):91-101.
The mammalian hippocampus has been the focus of several neurobiology studies because of its important behavioral function and because long-term potentiation (LTP) is a prominent feature of this brain region. Converging evidence suggests that hippocampal function is associated with learning multiple relationships of environmental cues. In this paper a novel behavioral test procedure is introduced, a modified T-maze continuous alternation task (T-CAT), that may serve as a simple, automatable, and quick test of hippocampal function in addition to the frequently applied water maze and fear conditioning paradigms. A comparison is made between mice (strain C57BL/6) with ibotenic acid lesioned or vehicle injected hippocampus, two transgenic strains (on CD1 background) overexpressing a calcium binding protein, S100beta, and inbred (C57BL/6, DBA/2, 129/SV and 129/SVEV) and outbred (CD1) strains of mice. This study shows that hippocampal lesioning led to a significant impairment in T-CAT. Furthermore, overexpression of S100beta, which impairs hippocampal LTP, also led to an impairment demonstrating that T-CAT is sensitive to detect hippocampal dysfunction. Analysis of the mouse strains revealed that C57BL/6 and CD1 mice performed well in T-CAT, whereas 129/SV, 129/SVEV and DBA/2 were significantly impaired, a finding that underscores the importance of strain differences in pharmacological or single gene manipulation studies of hippocampal function in mice.

Jacobsson SO, Fowler CJ. **Dopamine and glutamate neurotoxicity in cultured chick telencephali cells: effects of NMDA antagonists, antioxidants and MAO inhibitors.** Neurochem Int 1999;34(1):49-62.
In a recent study, it was found that the intrastriatal administration to rats of the organophosphorous compound soman and kainic acid produced a rapid release not only of glutamate but also of dopamine in this brain region. Dopamine is a potent source of free radicals and is known to produce cytotoxic effects, per se. This raises the possibility that the released glutamate and dopamine act synergistically to produce the neurotoxicity found after soman administration. In order to investigate the feasibility of this hypothesis in an in vitro system, the effects of dopamine and glutamate upon cell survival were investigated using chick neurons (7 DIV) in serum-free primary culture. The neurons were treated with dopamine and/or glutamate for up to 24 h and cell toxicity was then assessed either by determination of cell densities, by the release of cytoplasmic LDH or by the MTT cytotoxicity assay. L-Glutamate produced a concentration-dependent cytotoxicity that was seen as early as after 30 min of exposure, and was accompanied by an increased level of lipid peroxidation. The L-glutamate toxicity could to a large extent by prevented by NMDA receptor antagonists and to a lesser extent by catalase, superoxide dismutase or glutathione ethyl ester added 30 min before the glutamate. Dopamine was also cytotoxic, and the cytotoxicity was reduced by the combination of catalase and glutathione ethyl ester but not by the MAO inhibitors clorgyline or L-deprenyl, or by the selective dopamine uptake inhibitor GBR 12783. The cytotoxic effects of dopamine and L-glutamate were additive rather than synergistic, regardless of the incubation time used. It is concluded that chick neurons in serum-free culture are a useful in vitro

model system for the study of cell toxicity produced by oxidative stress and by glutamate. The cytotoxic effects of dopamine in this model are not due to the monoamine oxidase-mediated production of hydrogen peroxide but appear at least in part to be related to oxidative stress.

Jiang Z, Carlin KP, Brownstone RM. **An in vitro functionally mature mouse spinal cord preparation for the study of spinal motor networks.** Brain Res 1999;816(2):493-9.
An in vitro isolated whole spinal cord preparation has been developed in 'motor functionally mature' mice; that is mice of developmental maturity sufficient to weight-bear and walk. In balb/c mice this stage occurs at around postnatal day 10 (P10). Administration of strychnine elicited synchronous activity bilaterally in lumbar ventral roots. Rhythmic alternating locomotor-like activity could be produced by application of a combination of serotonin (5-HT), N-methyl-d-aspartate (NMDA), and dopamine in animals up to P12. Using a live cell-dead cell assay, it is demonstrated that there are primarily viable cells throughout the lumbar spinal cord. The viability of descending pathways was demonstrated with stimulation of the mid-thoracic white matter tracts. In addition, polysynaptic segmental reflexes could be elicited. Although usually absent in whole cord preparations, monosynaptic reflexes could invariably be elicited following longitudinal midline hemisection, leading to the possible explanation that there might be an active crossed pathway producing presynaptic inhibition of primary afferent terminals. The data demonstrate that this functionally mature spinal cord preparation can be used for the study of spinal cord physiology including locomotion. Copyright 1999 Elsevier Science B.V.

Liu J, Morrow AL, Devaud L, Grayson DR, Lauder JM. **GABAA receptors mediate trophic effects of GABA on embryonic brainstem monoamine neurons in vitro.** J Neurosci 1997;17(7):2420-8.
The inhibitory neurotransmitter GABA may act as a trophic signal for developing monoamine neurons in embryonic rat brain, because GABA neurons and their receptors appear in brainstem during generation of monoamine neurons. To test this hypothesis, we used dissociated cell cultures from embryonic day 14 rat brainstem, which contains developing serotonin (5-HT), noradrenaline (tyrosine hydroxylase; TH), and GABA neurons. Immunocytochemistry and reverse transcription-PCR (RT-PCR) revealed the presence of multiple alpha, beta, gamma, and delta subunits in these cultures. Competitive RT-PCR demonstrated high levels of beta3 subunit transcripts. Expression of functional GABAA receptors was demonstrated using 36Cl- flux assays. To investigate GABAergic regulation of neuronal survival and growth, cultures were treated for 1-3 d in vitro with 10 microM GABA and/or GABAA antagonist (bicuculline or the pesticide dieldrin). The effects of treatments were quantified by analysis of immunoreactive 5-HT, TH, and GABA neurons. GABAA receptor ligands differentially regulated neuronal survival and growth depending on neurotransmitter phenotype. GABA exerted positive effects on monoamine neurons, which were countered by bicuculline (and dieldrin, 5-HT neurons only). By itself, bicuculline produced inhibitory effects on both 5-HT and TH neurons, whereas dieldrin potently inhibited 5-HT neurons only. GABA neurons responded positively to both antagonists, but more strongly to bicuculline. Taken together, these results demonstrate that the activation/inhibition of GABAA receptors produces opposite effects on the development of embryonic monoamine and GABA neurons. This suggests that these neurotransmitter phenotypes may express GABAA receptors that differ in fundamental ways, and these differences determine the developmental responses of these cells to GABAergic stimuli.

MClean WG, Ward SA. **In vitro neurotoxicity of artemisinin derivatives.** Med Trop (Mars) 1998;58 (3 Suppl):28-31.
The known neurotoxicity of high doses of arteether and dihydroartemisinin in experimental animals has led to the need for a rapid screening method to predict the potential neurotoxicity of newly developed artemisinin-related antimalarial drugs. We have studied the effects of a range of these compounds on the neurite outgrowth of differentiating NB2a neuroblastoma cells in vitro, an assay that shows a correlation with neurotoxicity in vivo for a range of neurotoxic agents. In this assay, dihydroartemisinin is significantly more toxic than artemether or arteether. In the presence of liver metabolising enzymes, in vitro neurotoxicity of artemether and arteether is markedly increased. Differentiated neuronal cells are more sensitive than differentiated glial cells. Electron microscopy confirms that the targets in the neuronal cell for dihydroartemisinin are mitochondrial membranes and endoplasmic reticulum. The technique forms a valuable component of a range of appropriate neurotoxicity screening tests that should continue to be applied to newly developed antimalarials of this type.

Morris EJ, Dreixler JC, Cheng KY, Wilson PM, Gin RM, Geller HM. **Optimization of single-cell gel electrophoresis (SCGE) for quantitative analysis of neuronal DNA damage.** Biotechniques 1999;26 (2):282-9.

Rego AC, Areias FM, Santos MS, Oliveira CR. **Distinct glycolysis inhibitors determine retinal cell sensitivity to glutamate-mediated injury.** Neurochem Res 1999;24(3):351-8.
In this study, we analyzed how distinct glycolysis inhibitors influenced the redox status of retinal cells, used as a neuronal model. Three different approaches were used to inhibit glycolysis: the cells were submitted to iodoacetic acid (IAA), an inhibitor of glyceraldehyde 3-phosphate dehydrogenase, to 2-deoxy-glucose (DG) in glucose-free medium, which was used as a substitute of glucose, or in the absence of glucose. The redox status of the cells was evaluated by determining the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide). By the analysis of dose-response curves of MTT reduction, IAA showed values of IC50 = 7.02 x 10(-5) M, whereas DG showed values of IC50 = 7.42 x 10(-4) M. Upon 30 min-incubation, glucose deprivation, per se, did not significantly affect MTT reduction. We also evaluated the reduction of MTT as an indicator of cell injury by exposing the cells to 100 microM glutamate during the decrement of glycolysis function. In the presence of glutamate, for 2 h, there was a decrease in MTT reduction, which was potentiated in the presence of DG (10-20% decrease), in the presence of IAA (about 30% decrease) or in glucose-free medium (about 30% decrease). Major changes observed by the MTT assay, upon exposure to glutamate, indicative of changes in the redox status of retinal cells, were concomitant with variations in intracellular ATP. Under glucose deprivation, endogenous ATP decreased significantly from 38.9+/-4.4 to 13.3+/-0.7 nmol/mg protein after exposure to 100 microM glutamate. The results support a different vulnerability of retinal cells after being exposed to distinct forms of glycolysis inhibition.

Varona A, Echevarria E, Irazusta J, Serrano R, Gil J, Casis L. **Effects of acute benzene exposure on brain enkephalin immunostaining and degradation.** Neurotoxicol Teratol 1998;20(6):611-6.
Benzene is a widely used industrial neurotoxin. Its action in the brain is not completely clear. Acute exposure to benzene in vivo reduces aminopeptidase activity in the hypothalamus and in the brain stem, and in vitro treatment with this toxicant generates a dose-related inhibition of this enzyme, notably in the

brain stem. Enkephalin immunostaining shows a significant increase in substantia nigra, central gray (dorsal, lateral and pontine), and tegmental nuclei in acute in vivo benzene-treated rats with respect to controls. In summary, this work shows an effect of benzene on the enkephalinergic system, which could be related to the naloxone-reversible analgesia induced by the aromatic hydrocarbon.

OCULAR TOXICITY

Bruner LH, Evans MG, Mcpherson JP, Southee JA, Williamson PS. **Investigation of ingredient interactions in cosmetic formulations using isolated bovine corneas.** Toxicol In Vitro 1998;12 (6):669-90.
BIOSIS COPYRIGHT: BIOL ABS. The purpose of this paper is to report on use of a modified bovine cornea opacity and permeability assay (BCOP) to test the effects of several cosmetic formulations on eye-derived tissue in vitro. The results from these studies suggest that a BCOP protocol using prolonged exposure and repeated treatments may be useful for screening the eye effects of cosmetic formulations. Further work will be required, however, before the model is ready for formal validation. This series of experiments also provides an example of where the toxicity of one ingredient was significantly changed by its interaction with other ingredients in a mixture. As it was not possible to predict the highly reactive nature of the formulation in vitro based on an evaluation of ingredient toxicity data alone, this case illustrates the importance of obtaining adequate safety testing data on innovative mixtures of cosmetic ingredients before human exposure is allowed.

Cho KS, Lee EH, Choi JS, Joo CK. **Reactive oxygen species-induced apoptosis and necrosis in bovine corneal endothelial cells.** Invest Ophthalmol Vis Sci 1999;40(5):911-9.
PURPOSE: The loss of corneal endothelial cells associated with aging and possibly other causes has been speculated to be related to exposure to reactive oxygen species (ROS). The current study was conducted to investigate, by use of photosensitizers, the underlying mechanisms involved in the death of bovine corneal endothelial cells (BCENs) caused by ROS. METHODS: BCEN cells in primary culture were treated with a photosensitizer (riboflavin or rose bengal) with light exposure. The patterns of cell damage and death were assessed using an acridine orange-ethidium bromide differential staining method, TdT-mediated dUTP nick-end labeling (TUNEL) assay, and transmission electron microscopy. The cytotoxicity was assayed by mitochondrial function using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) testing. Antioxidants, including catalase, L-histidine, salicylic acid, and superoxide dismutase, were used to determine the types of ROS involved. Activation of nuclear factor (NF)-kappaB was examined by fluorescent immunocytochemistry with anti-p65 antibody. RESULTS: Light-irradiated riboflavin or rose bengal resulted in a significant decrease in viability of BCEN cells. Chromosomal condensation and fragmentation were observed in apoptotic cells, and membrane lysis and damage of cell ultrastructures were observed in necrotic cells. Riboflavin induced apoptosis at 30 minutes and thereafter and induced necrosis after 2 hours. Rose bengal was shown to cause similar effects within half the time required for the effects of riboflavin. Catalase and salicylic acid were found to provide protection for BCENs from cytotoxic effects of riboflavin, and L-histidine was found to protect BCENs from cytotoxicity induced by rose bengal. Kinetic studies using immunocytochemistry showed that NF-kappaB was translocated into the nucleus within 15 minutes and 30 minutes after treatment with rose bengal and riboflavin, respectively. CONCLUSIONS: The cytotoxic effects of photo-

irradiated riboflavin and rose bengal are shown to be mediated by two distinct but parallel pathways, one leading to apoptosis and the other to necrosis. Possible involvement of NF-kappaB in cell death is suggested. These findings provide potential leads for future investigation into the molecular mechanisms of loss of corneal endothelial cells related to aging, oxidative stress, and possibly other similar causes.

Goskonda VR, Khan MA, Hutak CM, Reddy IK. **Permeability characteristics of novel mydriatic agents using an in vitro cell culture model that utilizes SIRC rabbit corneal cells.** J Pharm Sci 1999;88(2):180-4.
The purpose of this study was to evaluate the permeability characteristics of a previously reported in vitro corneal model that utilizes SIRC rabbbit corneal cells and to investigate the permeability of three novel esters of phenylephrone chemical delivery systems (CDS) under different pH conditions using this in vitro model. The SIRC rabbit corneal cell line was grown on transwell polycarbonate membranes, and the barrier properties were assessed by measuring transepithelial electrical resistance (TEER) using a voltohmmeter. The permeabilities of esters of phenylephrone CDS across the SIRC cell layers were measured over a pH range 4.0-7. 4. The esters tested include phenylacetyl (1), isovaleryl (2), and pivalyl (3). The SIRC rabbit corneal cell line, when grown on permeable filters, formed tight monolayers of high electrical resistance with TEER values increasing from 71.6 +/- 20.8 Omega.cm2 at day 3 in culture to 2233.42 +/- 15.2 Omega.cm2 at day 8 in culture and remained constant through day 14 in culture. The transepithelial permeability coefficients (Papp) at pH 7.4 ranged from 0.58 x 10(-6) cm/s for the hydrophilic marker, mannitol, to 43. 5 x 10(-6) cm/s for the most lipophilic molecule, testosterone. The Papp at pH 7.4 for phenylephrine was 4.21 x 10(-6) cm/s. The Papp values and the lag times of the three esters of phenylephrone were pH dependent. The Papp for 1, 2, and 3 at pH 7.4 were 14.76 x 10(-6), 13.19 x 10(-6), and 12.86 x 10(-6) cm/s, respectively and the permeabilities decreased at conditions below pH 7.4. The lag times at pH 7.4 were 0.10, 0.17, and 0.12 h for 1, 2, and 3, respectively, and the values increased at lower pH conditions. The TEER values of SIRC cell line observed at day 8 to day 14 in the present investigation are similar to the resistance value reported for rabbit cornea (2 kOmega. cm2). All the esters showed significantly ($p < 0.05$) higher permeabilities than phenylephrine at pH 7.4. The rate and extent of transport of the drugs across the cell layers were influenced by the fraction of ionized and un-ionized species and the intrinsic partition coefficient of the drug. The results indicate that the permeability of ophthalmic drugs through ocular membranes may be predicted by measuring the permeability through the new in vitro cell culture model.

Hueber A, Esser P, Heimann K, Kociok N, Winter S, Weller M. **The topoisomerase I inhibitors, camptothecin and beta-lapachone, induce apoptosis of human retinal pigment epithelial cells.** Exp Eye Res 1998;67(5):525-30.
The aim of the study was to determine whether the topoisomerase I inhibitors, camptothecin and beta-lapachone, are suitable agents for the adjuvant pharmacotherapy of proliferative vitreoretinopathy (PVR). The effects of the drugs on cultured human retinal pigment epithelial (RPE) cells were examined using growth assays, cytotoxicity assays, single cell agarose gel electrophoresis, in situ DNA end labeling and immunoblot analysis for apoptosis-regulatory proteins. Both agents killed RPE cells in a concentration-and time-dependent manner. Cell death was apoptotic as assessed by single cell agarose gel electrophoresis and in situ DNA end labeling. Camptothecin, but not beta-lapachone, induced accumulation of p53 and the major growth arrest-associated p53 response protein, p21. Both drugs

enhanced expression of the proapoptotic BAX protein. Camptothecin, but not beta-lapachone, synergistically enhanced RPE cell apoptosis induced by the cytotoxic cytokine, CD95 ligand (CD95L). This effect was linked to camptothecin-induced inhibition of RNA synthesis. Atypical topoisomerase I inhibitors may be promising agents for the adjuvant pharmacotherapy of PVR. Experimental studies to assess possible ocular toxicity upon local administration and to confirm its therapeutic efficacy in an animal model of PVR are required. Copyright 1998 Academic Press.

Pham XT, Huff JW. **Cytotoxicity evaluation of multipurpose contact lens solutions using an in vitro test battery.** CLAO J 1999;25(1):28-35.
PURPOSE: Many in vitro alternatives to eye irritation testing are not mechanism-specific and do not employ ocular cell lines. We have developed an effective and reliable test battery that reveals toxicity mechanisms of contact lens solutions on cell metabolism and proliferation. METHODS: Cytotoxicity endpoints were quantified using bovine corneal epithelial cultures in 96-well microplates. A kenacid blue assay provided information on total cell protein, while lactate production and alamarBlue assays served as indicators of aerobic/anaerobic metabolism and redox state of cells grown in serum-free Dulbecco's modified Eagle's/Ham's F12 medium (DMEM/F12). Concentrations (% v/v) causing 10-90% inhibition of the control assay responses were used for correlations with in vivo data. RESULTS: Cytotoxicities of contact lens solutions correlated better with irritant symptoms than with corneal staining, and were ranked as follows: Lens Plus << Opti-Free < or = ContaClair < or = ReNu. Lens Plus was not toxic to cell glycolysis, respiration, and proliferation for up to 20% v/v. However, the multi-purpose solutions inhibited these endpoints in a concentration-dependent manner. Opti-Free and ReNu, containing Dymed and Polyquad (ammonium surfactants), showed non-specific cell inhibition. The lactate production assay had a flatter log concentration-response curve than the other two assays. CONCLUSIONS: The proposed biochemically-based test battery using the target corneal epithelium has the potential to be a simple and effective method for screening and defining toxicity profiles of contact lens care solutions. The model can be applicable to small- or large-scale testing programs and research and development of new ocular products.

Saarinen-Savolainen P, Jarvinen T, Araki-Sasaki K, Watanabe H, Urtti A. **Evaluation of cytotoxicity of various ophthalmic drugs, eye drop excipients and cyclodextrins in an immortalized human corneal epithelial cell line**. Pharm Res 1998 Aug;15:1275-80.
IPA COPYRIGHT: ASHP An immortalized human corneal epithelial cell line used as a screening tool for prediction of topical ocular irritation was tested. Mitochondrion-based MTT test was a more sensitive indicator of cytotoxicity than the plasma membrane based propidium iodide test. Cytotoxic rankings were made for in vivo applied ophthalmic drugs, excipients, and for cyclodextrins. Toxic effects were seen after short exposure (5 min) and toxic effects of some test substances were seen only after longer exposure times (30 and 60 min).

PHARMACOKINETIC AND MECHANISTIC STUDIES

Antonsson B, Marshall CJ, Montessuit S, Arkinstall S. **An in vitro 96-well plate assay of the mitogen-activated protein kinase cascade.** Anal Biochem 1999;267(2):294-9.
Mitogen-activated protein (MAP) kinases of the extracellular signal-regulated kinase (ERK) family are

activated in response to many growth and differentiation factors as well as some oncogenes. ERK activation follows phosphorylation by a class of specific upstream MAP kinase/ERK kinase (MEK) exemplified by MEK-1. Activated ERKs control many short- and long-term changes in cell function through phosphorylating a number of intracellular target substrates which include stathmin, a phosphoprotein regulating microtubule stability. We report here the development of a simple, 96-well plate, quantitative in vitro assay measuring purified ERK2 catalytic activation by a constitutive MEK-1 mutant (S218E S222E). Enzymatic activity was detected by 33P phosphorylation of purified biotinylated stathmin captured on streptavidin-coated scintillation proximity assay beads which eliminates the need for wash steps. The assay was optimized and the K0.5 value for ATP was found to be 0.9 microM and the Km for stathmin was determined to be 16 microM. The assay was also used to determine IC50 values for the protein kinase inhibitors PD98059 and staurosporine. This simple assay allows several hundred quantitative measurements of MEK1-dependent ERK2 activation to be performed in a day.

Ashton M, Johansson L, Thornqvist AS, Svensson US. **Quantitative in vivo and in vitro sex differences in artemisinin metabolism in rat.** Xenobiotica 1999;29(2):195-204.
1. The pharmacokinetics of the antimalarial compound artemisinin were compared in the male and female Sprague-Dawley rat after single dose i.v. (20 mg x kg(-1)) or i.p. (50 mg x kg(-1)) administration of an emulsion formulation. 2. Plasma clearance of artemisinin was 12.0 (95% confidence interval: 10.4, 13.0) 1 x h(-1) x kg(-1) in the male rat and 10.6 (95% CI: 7.5, 15.0) 1 x h(-1) x kg(-1) in the female rat suggesting high hepatic extraction in combination with erythrocyte uptake or clearance. Artemisinin half-life was approximately 0.5 h after both routes of administration in both sexes. Values for plasma clearance and half-lives did not statistically differ between the sexes. 3. After i.p. administration artemisinin AUCs were 2-fold higher in the female compared with male rat ($p < 0.001$). Artemisinin disappearance was 3.9-fold greater in microsomes from male compared with female livers and it was inhibited in male microsomes by goat or rabbit serum containing antibodies against CYP2C11 and CYP3A2 but not CYP2B1 or CYP2E1. 4. The unbound fraction of artemisinin in plasma was lower ($p < 0.001$) in plasma obtained from the male (8.8 +/- 2.0%) compared with the female rat (11.7 +/- 2.2%). 5. The possibility of a marked sex difference, dependent on the route of administration, has to be taken into account in the design and interpretation of toxicological studies of artemisinin in this species.

Bachman J, Patterson HH. **Photodecomposition of the carbamate pesticide carbofuran: Kinetics and the influence of dissolved organic matter.** Environ Sci Technol 1999;33(6):874-81.
 This study examined the photodecomposition of carbofuran, a carbamate pesticide with high oral toxicity. Rate constants are measured for the pesticide in aqueous solution and in the presence of various samples of dissolved organic matter (DOM). Kinetic experiments are monitored with HPLC, while reaction products are determined using HPLC, GC-MS, and 1H NMR: mechanisms are proposed for the first three steps of the reaction. It was found that the photodecomposition proceeds via first-order reactio carbamate group is cleaved from the molecule. The furan moiety is opened in the second step producing a substituted catechol with a tert-butyl alcohol group as the substituent at the number three carbon. This molecule then undergoes a dehydration reaction to form an alkene side group from the tert-butyl alcohol side group.

Barber D, Correll L, Ehrich M. **Comparison of two in vitro activation systems for protoxicant organophosphorous esterase inhibitors.** Toxicol Sci 1999;47(1):16-22.
In order to perform in vitro testing of esterase inhibition caused by organophosphorous (OP) protoxicants, simple, reliable methods are needed to convert protoxicants to their esterase-inhibiting forms. Incubation of parathion or chlorpyrifos with 0.05% bromine solution or uninduced rat liver microsomes (RLM) resulted in production of the corresponding oxygen analogs of these OP compounds and markedly increased esterase inhibition in SH-SY5Y human neuroblastoma cells. Neither activation system affected cell viability or the activity of AChE or NTE in the absence of OP compounds. Although parathion and chlorpyrifos were activated by RLM, bromine activation required fewer steps and produced more esterase inhibition for a given concentration of chlorpyrifos. However, RLM activation of OP protoxicants produced metabolites other than oxygen analogs and may, therefore, be more relevant as a surrogate for OP biotransformation in vivo. This methodology makes the use of intact cells for in vitro testing of esterase inhibition caused by protoxicant organophosphate compounds a viable alternative to in vivo tests.

Basar T, Havlicek V, Bezouskova S, Halada P, Hackett M, Sebo P. **The conserved lysine 860 in the additional fatty-acylation site of Bordetella pertussis adenylate cyclase is crucial for toxin function independently of its acylation status.** J Biol Chem 1999;274(16):10777-83.
The Bordetella pertussis RTX (repeat in toxin family protein) adenylate cyclase toxin-hemolysin (ACT) acquires biological activity upon a single amide-linked palmitoylation of the epsilon-amino group of lysine 983 (Lys983) by the accessory fatty-acyltransferase CyaC. However, an additional conserved RTX acylation site can be identified in ACT at lysine 860 (Lys860), and this residue becomes palmitoylated when recombinant ACT (r-Ec-ACT) is produced together with CyaC in Escherichia coli K12. We have eliminated this additional acylation site by replacing Lys860 of ACT with arginine, leucine, and cysteine residues. Two-dimensional gel electrophoresis and microcapillary high performance liquid chromatography/tandem mass spectrometric analyses of mutant proteins confirmed that the two sites are acylated independently in vivo and that mutations of Lys860 did not affect the quantitative acylation of Lys983 by palmitoyl (C16:0) and palmitoleil (cis Delta9 C16:1) fatty-acyl groups. Nevertheless, even the most conservative substitution of lysine 860 by an arginine residue caused a 10-fold decrease of toxin activity. This resulted from a 5-fold reduction of cell association capacity and a further 2-fold reduction in cell penetration efficiency of the membrane-bound K860R toxin. These results suggest that lysine 860 plays by itself a crucial structural role in membrane insertion and translocation of the toxin, independently of its acylation status.

Behnam-Motlagh P, Engstrom KG, Henriksson R, Grankvist K. **Serotoninergic modulation of cell volume response to estramustine: an image-analysis study on perifused individual glioma cells.** Eur J Cancer 1999;35(1):145-53.
A technique of microscopy with computerised detection of early morphological changes during continuous perifusion was used to monitor the geometry changes of cultured glioma cells (MG-251) when exposed to 40 mg/L estramustine phosphate (EMP) alone or in combination with granisetron (0.1 mumol/L), ondansetron (0.1 mumol/L), or serotonin (1 mumol/L). When the cells were exposed to EMP, cell volume measured as projected cell area (PCA) rapidly increased. Serotonin and ondansetron, but not granisetron, prevented the acute EMP response (PCA). Serotonin, but none of the 5-HT3 receptor

antagonists, protected against the cytotoxicity of EMP to the glioma cells as measured by a fluorometric microculture assay. Our results demonstrate hitherto unknown differences between selective 5-HT3 receptor antagonist on the cellular response to EMP and shows the necessity to study the receptor antagonists from viewpoint of interference with the antitumour drug effects on malignant cells. The perifusion technique could be used to study the effects of serotoninergic agonists and antagonists on cell volume regulation of cells exposed to anticancer drugs.

Bergman K, Olofsson I, Sjoberg P. **Dose selection for carcinogenicity studies of pharmaceuticals: systemic exposure to phenacetin at carcinogenic dosage in the rat.** Regul Toxicol Pharmacol 1998;28 (3):226-9.
A systemic exposure-based alternative to the MTD (maximally tolerated dose) for high-dose selection in carcinogenicity studies of pharmaceuticals has been accepted by the ICH (International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use). As a result of a retrospective analysis performed by the U.S. FDA (United States Food and Drug Administration), a rat/human relative systemic exposure ratio of 25 is proposed by the ICH as an acceptable pharmacokinetic endpoint for high-dose selection. For use as a dose selection criterion, it is particularly important that the magnitude of the relative systemic exposure ratio should be sufficient to detect human pharmaceuticals classified by IARC (International Agency for Research on Cancer, World Health Organization) as known (category 1) or probable (category 2A) human carcinogens. For one of these, phenacetin (an IARC 2A compound and a rat carcinogen), a systemic exposure ratio of 15 was calculated by the FDA. This calculation was based on a number of extrapolations. The present study reports the actual systemic exposure to phenacetin in the rat under conditions mimicking the conditions in the carcinogenicity study used by the FDA to calculate the relative systemic exposure ratio of 15. The ratio was found to be 7, indicating that the carcinogenic potential of this particular probable human carcinogen could be detected at a considerably lower systemic exposure ratio than that proposed by the ICH. Copyright 1998 Academic Press.

Bogdanffy MS, Sarangapani R, Kimbell JS, Frame SR, Plowchalk DR. **Analysis of vinyl acetate metabolism in rat and human nasal tissues by an in vitro gas uptake technique.** Toxicol Sci 1998;46 (2):235-46.
Physiologically based pharmacokinetic (PBPK) models require estimates of catalytic rate constants controlling the metabolism of xenobiotics. Usually, these constants are derived from whole tissue homogenates wherein cellular architecture and enzyme compartmentation are destroyed. Since the nasal cavity epithelium is composed of a heterogeneous cell population measurement of xenobiotic metabolizing enzymes using homogenates could yield artifactual results. In this article a method for measuring rates of metabolism of vinyl acetate, a metabolism-dependent carcinogen, is presented that uses whole-tissue samples and PBPK modeling techniques to estimate metabolic kinetic parameters in tissue compartments. The kinetic parameter estimates were compared to those derived from homogenate experiments using two methods of tissue normalization. When the in vitro gas uptake constants were compared to homogenate-derived values, using a normalization procedure that does not account for tissue architecture, there was poor agreement. Homogenate-derived values from rat nasal tissue were 3- to 23-fold higher than those derived using the in vitro gas uptake method. When the normalization procedure for the rat homogenate-derived values took into account tissue architecture, a good agreement

was observed. Carboxylesterase activity in homogenates of human nasal tissues was undetectable. Using the in vitro gas uptake technique, however, carboxylesterase activity was detected. Rat respiratory carboxylesterase and aldehyde dehydrogenase activities were about three and two times higher than those of humans, respectively. Activities of the rat olfactory enzymes were about equivalent to those of humans. K(m) values did not differ between species. The results suggest that the in vitro gas uptake technique is useful for deriving enzyme kinetic constants where effects of tissue architecture are preserved. Furthermore, the results suggest that caution should be exercised when scaling homogenate-derived values to whole-organ estimates, especially in organs of cellular heterogeneity.

Calabrese CR, Loadman PM, Lim LS, Bibby MC, Double JA, Brown JE, Lamb JH. **In vivo metabolism of the antitumor imidazoacridinone C1311 in the mouse and in vitro comparison with humans.** Drug Metab Dispos 1999;27(2):240-5.
C1311 has emerged as the lead compound from a novel group of anticancer agents, the imidazoacridinones, and will be entering clinical trials shortly. Previous murine pharmacokinetic studies have shown C1311 to be rapidly and extensively distributed into tissues including tumor. This study has identified two major metabolites of C1311 and describes their pharmacokinetics in mice. M1 is a glucuronide of the parent compound with high concentrations in both plasma and liver. Calculated area under the plasma concentration versus time curve values were 6-fold and 2-fold greater, respectively, than C1311. Based on these studies, we propose M2 to be a nonfluorescent oxidation product because electrospray ionization-mass spectroscopy/mass spectroscopy analysis gave a molecular ion at m/z 367, 16 U greater than the parent compound. It formed rapidly in liver preparations in vitro, both murine and human, by a cytosolic process in the presence of NADPH and in vivo was detected in liver tissues at concentrations equivalent to those of C1311 but was not detectable in plasma. Preliminary in vitro toxicity studies showed M2 to be as potent as C1311 against MAC15A tumor cells. Over the first 24 h, 39% of the administered dose is eliminated via the bile (28%) mostly as C1311 or the kidneys (11%) as the glucuronide (M1). This study has given valuable information as to the likely metabolic pathway to occur in humans, and the cytotoxic metabolite M2 may play a role in the antitumor activity or toxicity of C1311 in the clinic.

Campos-Caro A, Carrasco-Serrano C, Valor LM, Viniegra S, Ballesta JJ, Criado M. **Multiple functional Sp1 domains in the minimal promoter region of the neuronal nicotinic receptor alpha5 subunit gene.** J Biol Chem 1999;274(8):4693-701.
The alpha5 subunit is a component of the neuronal nicotinic acetylcholine receptors, which are probably involved in the activation step of the catecholamine secretion process in bovine adrenomedullary chromaffin cells. The promoter of the gene coding for this subunit was isolated, and its proximal region was characterized, revealing several GC boxes located close to the site of transcription initiation (from -111 to -40). Deletion analysis and transient transfections showed that a 266-base pair region (-111 to +155) gave rise to approximately 77 and 100% of the maximal transcriptional activity observed in chromaffin and SHSY-5Y neuroblastoma cells, respectively. Site-directed mutagenesis of five different GC motifs indicated that all of them contribute to the activity of the alpha5 gene, but in a different way, depending on the type of transfected cell. Thus, in SHSY-5Y cells, alteration of the most promoter-proximal of the GC boxes decreased alpha5 promoter activity by approximately 50%, whereas single mutations of the other GC boxes had no effect. In chromaffin cells, by contrast, modification of any of

the GC boxes produced a similar decrease in promoter activity (50-69%). In both cell types, however, activity was almost abolished when four GC boxes were suppressed simultaneously. Electrophoretic mobility shift assays using nuclear extracts from either chromaffin or SHSY-5Y cells showed the specific binding of Sp1 protein to fragment -111 to -27. Binding of Sp1 to the GC boxes was also demonstrated by DNase I footprint analysis. This study suggests that the general transcription factor Sp1 plays a dominant role in alpha5 subunit expression, as has also been demonstrated previously for alpha3 and beta4 subunits. Since these three subunits have their genes tightly clustered and are expressed in chromaffin cells, probably as components of the same receptor subtype, we propose that Sp1 constitutes the key factor of a regulatory mechanism common to the three subunits.

Catret M, Anselmi E, Ivorra MD, Elorriaga M, Tur R, D'Ocon MP. **Alpha-adrenoceptor interaction of tetrandrine and isotetrandrine in the rat: functional and binding assays.** J Pharm Pharmacol 1998;50 (11):1267-73.
The action of 1S,1'S-tetrandrine, a bisbenzyltetrahydroisoquinoline alkaloid, on alpha1-adrenoceptors has been compared with that of its isomer 1R,1'S-isotetrandrine. The work includes binding assays to analyse the affinity of these products for the [3H]prazosin binding site of rat cerebral cortical membranes and functional studies on rat isolated aorta to examine the effects of both alkaloids on intracellular calcium processes related or not to alpha-adrenoceptor activation. A radioligand receptor-binding study showed that both compounds interacted with the alpha1-adrenoceptors displacing [3H] prazosin from the specific binding site. The Ki values (inhibition constants) were 0.69+/-0.12 and 1.6+/-0.4 microM for tetrandrine and isotetrandrine, respectively. The functional studies showed that both alkaloids concentration-dependently inhibited noradrenaline-induced contraction in Ca2+-free solution (IC50 values, i.e. the concentrations needed to induce 50% inhibition, were 252.8 and 174.9 microM for tetrandrine and isotetrandrine, respectively), the spontaneous contractile response elicited by extracellular calcium after depletion of noradrenaline-sensitive intracellular stores (increase in resting tone; IC50 values 11.6 and 19.6 microM for tetrandrine and isotetrandrine, respectively) and the refilling of intracellular Ca2+ stores sensitive to noradrenaline (IC50 values 7.4 and 14.9 microM for tetrandrine and isotetrandrine, respectively). The results show that tetrandrine and isotetrandrine interact with alpha1-adrenoceptors by displacing the [3H]prazosin binding site and that both compounds inhibit mainly the Ca2+-dependent process and have less action on alpha1-adrenoceptors. Tetrandrine is more potent than isotetrandrine.

Chomarat P, Rice JM, Slagle BL, Wild CP. **Hepatitis B virus-induced liver injury and altered expression of carcinogen metabolising enzymes: the role of the HBx protein.** Toxicol Lett 1998;102-103:595-601.
Hepatitis B virus (HBV) and aflatoxins are major risk factors for hepatocellular carcinoma (HCC) exhibiting a synergistic interaction in the development of this disease. The molecular mechanisms of this interaction remain to be elucidated but an altered carcinogen metabolism in the presence of hepatitis-induced liver injury is one hypothesis. The availability of biomarkers of aflatoxin exposure and metabolism permits this hypothesis to be examined in human populations whilst animal models, such as HBV transgenic mice permit parallel studies in an experimental setting. The hepatitis B virus X protein (HBx) is suspected to play a role in the hepatocarcinogenic process by virtue of its capacity to transactivate oncogenes and several other cellular genes via cis-acting elements. In previous studies in

HBV transgenic mice expressing the HB surface antigen and X genes we observed a marked induction of specific cytochrome P450s (CYP) (Kirby et al., 1994a). In the current study we investigated the status of CYP, glutathione S-transferases (GST) and antioxidant enzymes in mice carrying only the X gene under the control of the alpha-1 antitrypsin regulatory elements (ATX mice). Livers of ATX mice showed no major pathological alterations compared to age-matched non-transgenic control mice. Immunohistochemical staining for CYP1A, 2A5 and GST expression and determination of related enzymatic activities (7-ethoxyresorufin O-deethylation, 7-methoxyresorufin O-deethylation, coumarin 7-hydroxylation and GST activities) revealed no differences between control and ATX mice. In addition, no differences in antioxidant enzymes were observed. Overall, these results support the conclusion that HBx expression alone is insufficient to induce transactivation of CYP and GST genes or to alter the antioxidant system and that the induction in other HBV models is a result of inflammatory injury in the liver, a feature absent in ATX mice. These data are compared to biomarker studies of enzyme activities in aflatoxin-exposed human populations with and without HBV infection.

Collins AR, Olmedilla B, Southon S, Granado F, Duthie SJ. **Serum carotenoids and oxidative DNA damage in human lymphocytes.** Carcinogenesis 1998;19(12):2159-62.
Carotenoids are thought to act as antioxidants in vivo, decreasing oxidative damage to biomolecules and thus protecting against coronary heart disease and cancer. However, human intervention studies with beta-carotene have given equivocal results in terms of cancer incidence. In an alternative molecular epidemiological approach, we have employed the 'comet assay' (single cell alkaline gel electrophoresis) to measure strand breaks, oxidized pyrimidines and altered purines in the DNA of lymphocytes from volunteers supplemented with alpha/beta-carotene, lutein, lycopene or placebo. In addition, we measured concentrations of the main serum carotenoids, and vitamins E and C, by HPLC. We report a significant negative correlation between basal concentrations of total serum carotenoids and oxidized pyrimidines. A similar correlation was seen between individual carotenoids (notably lutein and beta-carotene) and oxidized pyrimidines. However, carotenoid supplementation did not have a significant effect on endogenous oxidative damage. This suggests that there are some factors in the basal diet, probably found in fruit and vegetables, that decrease oxidative damage to DNA. In this case, basal serum carotenoids may simply be markers of consumption of fruit and vegetables, they themselves having little or no protective value.

Curto EV, Kwong C, Hermersdorfer H, Glatt H, Santis C, Virador V, Hearing VJ Jr, Dooley TP. **Inhibitors of mammalian melanocyte tyrosinase: in vitro comparisons of alkyl esters of gentisic acid with other putative inhibitors.** Biochem Pharmacol 1999;57(6):663-72.
To discover safe and effective topical skin-lightening agents, we have evaluated alkyl esters of the natural product gentisic acid (GA), which is related to our lead compound methyl gentisate (MG), and four putative tyrosinase inhibitors, utilizing mammalian melanocyte cell cultures and cell-free extracts. Desirable characteristics include the ability to inhibit melanogenesis in cells (IC50 < 100 microg/mL) without cytotoxicity, preferably due to tyrosinase inhibition. Of the six esters synthesized, the smaller esters (e.g. methyl and ethyl) were more effective enzyme inhibitors (IC50 approximately 11 and 20 microg/mL, respectively). For comparison, hydroquinone (HQ), a commercial skin "bleaching" agent, was a less effective enzyme inhibitor (IC50 approximately 72 microg/mL), and was highly cytotoxic to melanocytes in vitro at concentrations substantially lower than the IC50 for enzymatic inhibition. Kojic

acid was a potent inhibitor of the mammalian enzyme (IC50 approximately 6 microg/mL), but did not reduce pigmentation in cells. Both arbutin and magnesium ascorbyl phosphate were ineffective in the cell-free and cell-based assays. MG at 100 microg/mL exhibited a minimal inhibitory effect on DHICA oxidase (TRP 1) and no effect on DOPAchrome tautomerase (TRP-2), suggesting that MG inhibits melanogenesis primarily via tyrosinase inhibition. MG and GA were non-mutagenic at the hprt locus in V79 Chinese hamster cells, whereas HQ was highly mutagenic and cytotoxic. The properties of MG in vitro, including (1) pigmentation inhibition in melanocytes, (2) tyrosinase inhibition and selectivity, (3) reduced cytotoxicity relative to HQ, and (4) lack of mutagenic potential in mammalian cells, establish MG as a superior candidate skin-lightening agent.

Darro F, Cahen P, Vianna A, Decaestecker C, Nogaret JM, Leblond B, Chaboteaux C, Ramos C, Petein M, Budel V, et al. **Growth inhibition of human in vitro and mouse in vitro and in vivo mammary tumor models by retinoids in comparison with tamoxifen and the RU-486 anti-progestagen.** Breast Cancer Res Treat 1998;51(1):39-55.
Retinoids constitute a very promising class of agents for the chemoprevention or treatment of breast cancer. These retinoids exert their biological activity through two distinct classes of retinoic acid (RA) receptors (R), the RAR isotypes (alpha, beta, and gamma) and the three RXR isotypes (alpha, beta, and gamma) and their numerous isoforms which bind as RXR/RAR heterodimers to the polymorphic cis-acting response elements of RA target genes. With respect to these numerous receptor sub-types, the retinoid-induced effects at the biological level include marked modifications with respect to both cell proliferation and cell death (apoptosis), and also in the induction of differentiation processes. The present study aims to characterize the effect which four retinoids (TTNPB, 9-cis-RA, LGD 1069, 4-HPR) with distinct RAR/RXR binding properties induced on various in vitro and in vivo mouse and human breast cancer models. The experiments with the retinoids were carried out in comparison with the anti-estrogen tamoxifen and the anti-progestagen RU-486 compounds. The results show that the 6 compounds under study were markedly more efficient in terms of growth inhibition in the human T-47D cell line when maintained under anchorage-independent culture conditions than when maintained under anchorage-dependent ones. While RU-486 exhibited a weak statistically significant ($p < 0.05$) influence on the growth of the T-47D stem cells, tamoxifen had a marked inhibitory influence on the growth of these cells. Of the four retinoids, 4-HPR was the least effective since the lowest doses tested (1 and 0.1 nM) exhibited no statistically ($p > 0.05$) significant influence on the growth of the stem cells. The most efficient retinoid was TTNPB. It was only at the highest dose (10 microM) that tamoxifen and RU-486 showed a weak inhibitory influence on the growth of the T-47D non-stem cells while all 4 retinoids exerted a significant inhibitory influence on the growth of these non-stem cells, with 4-HPR being the most efficient ($P < 0.001$) at the highest dose, but ineffective ($P > 0.05$) at the lowest. Tamoxifen and TTNPB were tested in vivo on hormone-sensitive (HS) and hormone-insensitive (HI) strains of the MXT murine mammary carcinoma. While TTNPB appeared to be equally efficient in terms of growth inhibition in both MXT-HS and MXT-HI models, tamoxifen had only a marginal inhibitory influence on the growth of the MXT-HI strain but did inhibit growth in the case of the MXT-HS one. TTNPB was markedly more efficient than tamoxifen in terms of both inhibiting the cell proliferation level (measured by means of computer-assisted microscopy applied to Feulgen-stained nuclei, a method which enables the percentage of cells in the S phase of the cell cycle to be determined) and triggering cell death (measured by means of the determination of the transglutaminase activity) in both the MXT-HI and

MXT-HS models. The very significant TTNPB-induced inhibition of the macroscopic MXT-HS growth rate relates to the triggering of cell death (apoptosis) rather than to an inhibition of cell proliferation. All these results clearly indicate that retinoids are very efficient agents against breast cancer, at least as efficient as tamoxifen.

Dekant W, Birner G, Werner M, Parker J. **Glutathione conjugation of perchloroethene in subcellular fractions from rodent and human liver and kidney.** Chem Biol Interact 1998;116(1-2):31-43.
Perchloroethene (Per) is a widely used industrial solvent and common environmental contaminant. In rats, long-term inhalation of Per is known to cause a small increase in the incidence of renal tubule cell tumors in males only; renal toxicity is seen in female rats and in both sexes of mice after prolonged Per exposure. The renal toxicity of Per is likely mediated by a glutathione-dependent bioactivation reaction. Glutathione S-transferase mediated formation of S-(1,2,2-trichlorovinyl)glutathione is the first step in a sequence of reactions finally resulting in the formation of reactive intermediates in the kidney. In this study, we compared the enzymatic rates of formation of S-(1,2,2-trichlorovinyl)glutathione in liver and kidney subcellular fractions from rats, mice, and from both sexes of humans (n = 11). In microsomal fractions from the liver and kidney of all three species, enzymatic formation of S-(1,2,2-trichlorovinyl) glutathione from Per could not be observed. S-(1,2,2-Trichlorovinyl)glutathione formation (the structure was confirmed by electrospray mass spectrometry) was observed in liver cytosol from both male and female rats and mice. However, the rates of S-(1,2,2-trichlorovinyl)glutathione formation in liver cytosol from male rats (84.5+/-12 pmol/mg per min) were approximately four times higher than from female rats (19.5+/-8 pmol/mg per min) and from both sexes of mice (27.9+/-6 and 26.0+/-4 pmol/mg per min). Low rates of S-(1,2,2-trichlorovinyl)glutathione formation were also seen in kidney cytosol from mice (12+/-6 pmol/mg per min), but not from rats. In human liver subcellular fractions, enzymatic formation of S-(1,2,2-trichlorovinyl)glutathione could not be detected. The human liver cytosolic fractions, however, exhibited glutathione S-transferase activity (as determined using 1-chloro-2,4-dinitrobenzene and hexachlorobutadiene as marker substrates) in the same order of magnitude as rat and mouse liver cytosol. In contrast to other marker activities for glutathione S-transferases, the ability of all human liver cytosol samples to catalyze the glutathione conjugation of 1,2-dichloro-4-nitrobenzene was three orders of magnitude lower compared to rat and mouse liver cytosol. 1,2-Dichloro-4-nitrobenzene conjugation was also four times higher in liver cytosol from male rats compared to female rats. The results suggest that the ability of the human liver to catalyze the formation of S-(1,2,2-trichlorovinyl)glutathione from Per is at least two orders of magnitude lower than that of rat liver, and that sex-specific differences in the extent of hepatic conjugation of Per with glutathione, which may be relevant for nephrotoxicity, occur in rats.

Drahushuk AT, Mcgarrigle BP, Slezak BP, Stegeman JJ, Olson JR. **Time- and concentration-dependent induction of CYP1A1 and CYP1A2 in precision-cut rat liver slices incubated in dynamic organ culture in the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin.** Toxicol Appl Pharmacol 1999;155(2):127-38.
In a previous 24-h study, precision-cut rat liver slices were validated as a useful in vitro model for assessing the dose-related induction of CYP1A1 and CYP1A2 in rat liver following exposure to 2, 3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Further assessment of the utility of this model was accomplished by initially exposing rat liver slices to medium containing TCDD (0.01 nM) for 24 h and incubating the

slices up to an additional 72 h in TCDD-free medium. The slices remained viable throughout the incubation period with an intracellular potassium content varying from 45.2 +/- 2.3 micromol/g at 48 h to 50.0 +/- 1.6 micromol/g at 72 h. In TCDD-exposed slices, CYP1A1 protein and its respective enzymatic activity, the O-deethylation of ethoxyresorufin (EROD), significantly increased with time over the 96-h incubation period, with EROD activity increasing from 63.6 +/- 14.2 at 24 h to 905 +/- 291 pmol/mg/min at 96 h. Under identical incubation conditions, but in the absence of TCDD, the EROD activity for the control liver slices ranged from 14. 3 +/- 4.3 to 44.9 +/- 11.9 pmol/min/mg. Conversely, the level of CYP1A2 protein and its respective activity (acetanilide hydroxylation) transiently decreased from 24 to 96 h with no significant differences observed between the control (0 nM TCDD) and treatment group (0.01 nM TCDD). The concentration-effect relationship at 96 h was characterized by incubating rat liver slices for the initial 24 h in medium containing TCDD at concentrations ranging from 0.1 pM to 10 nM. Induction of CYP1A1 protein and EROD activity was observed for all treatment groups with the 10 nM TCDD treatment group displaying greater than 100-fold induction compared to control (0 nM TCDD). Immunohistochemical localization of CYP1A1 protein within liver slices supported the time- and concentration-dependent induction of EROD activity by TCDD. The induction of CYP1A1 was initially observed to be centrilobular, with increased expression due to both elevated CYP1A1 within cells and the recruitment of additional cells expressing CYP1A1 throughout the entire liver slice. Additionally, the immunohistochemical analysis of the liver slices demonstrated the conservation of tissue architecture following up to 96 h of incubation in dynamic organ culture and provided further evidence for maintenance of tissue viability. In comparison to CYP1A1, the induction of CYP1A2 at 96 h was a less sensitive response, with significant induction of CYP1A2 protein and its respective activity occurring at a medium concentration of 0.1 nM TCDD (686 pg/g liver). In general, increasing the incubation period from 24 to 96 h markedly increased TCDD-induced expression of CYP1A1 and minimally enhanced CYP1A2 expression. Moreover, extending the incubation period to 96 h resulted in in vitro induction profiles for CYP1A1 and CYP1A2 that were qualitatively and quantitatively similar to that previously observed following in vivo exposure to TCDD (Drahushuk et al., Toxicol. Appl. Pharmacol. 140, 393-403, 1996). Copyright 1999 Academic Press.

Droz PO, Berode M, Jang JY. **Biological monitoring of tetrahydrofuran: contribution of a physiologically based pharmacokinetic model.** Am Ind Hyg Assoc J 1999;60(2):243-8.
A seven-compartment physiologically based pharmacokinetic (PBPK) model was developed to predict biological levels of tetrahydrofuran under various exposure scenarios. Affinities for the tissue were estimated from measurements of liquid-gas partition coefficients for water, olive oil, and blood. Metabolism was assumed to follow a rapid first order reaction. urinary excretion was simulated considering passive reabsorption of tetrahydrofuran in the tubules. The validity of the model was tested by comparison with available experimental and field data. Agreement was satisfactory with all studies available except one, which showed much higher results than expected. The source of this difference could not be identified, but cannot be explained by different exposure conditions, such as duration, concentration, or physical work load. However, it is recommended that this particular study not be used in the establishment of a biological exposure index. Simulation of repeated occupational exposure with the PBPK model allowed the prediction of biological levels that would be reached after repeated exposure at the American Conference of Governmental Industrial Hygienists' threshold limit value, time-weighted average of 200 ppm. For samples taken at the end of the shift, the PBPK model predicts 5.1

ppm for breath, 57 mumol/L (4.1 mg/L) for venous blood, and 100 mumol/L (7.2 mg/L) for urine.

Drumm K, Buhl R, Kienast K. **Additional NO2 exposure induces a decrease in cytokine specific mRNA expression and cytokine release of particle and fibre exposed human alveolar macrophages.** Eur J Med Res 1999;4(2):59-66.
Soot particles, asbestos fibres and irritant gas are common air pollutants which are able to induce lung and airway pulmonary injury. The aim of this study was to investigate the effect of a simultaneous NO2 and particle or fibre exposure on the proinflammatory specific mRNA expression and protein secretion of human alveolar macrophages (AM) in comparison to only particle or fibre exposed AM. AM were simultaneously exposed to FR 101, P 90, TiO2 or Chrysotile B at a concentration of 100 microg/10(6) cells and to NO2 at a concentration of 1.0 ppm for 30 min. Particle or fibre exposure of the AM was continued in humidified air at 5% CO2 and 37 degrees C for an additional hour (harvesting of total RNA) or additional 7 hrs (harvesting of culture supernatant). The mRNA expression of the proinflammatory cytokines IL-1beta, IL-6, IL-8 and TNF-alpha of NO2-particle/fibre co-exposed AM and only particle or fibre exposed AM was detected using specific RT-PCR. IL-1beta-, IL-6-, IL-8- and TNF-alpha-specific protein secretion was measured by ELISA. Cytotoxicity was detected by lactatedehydrogenase quantification in the culture supernatant. We observed an increased IL-1beta-, IL-6-, IL-8- and TNF-alpha-specific mRNA expression of particle or fibre exposed AM, which was decreased after an additional NO2 exposure. Also the particle or fibre exposure induced significant increase in IL-1beta-, IL-6-, IL-8 and TNF-alpha-release of AM which was decreased after an additional NO2 exposure ($p < 0.031$). The relative cytotoxicity of the NO2-particle/fibre co-exposure was higher than the particle or fibre induced cytotoxicity, but mostly <10%. Therefore it is concluded that particle or fibre exposure may result in an increase in proinflammatory cytokine release by AM, which may be decreased by toxic NO2 due to the oxidative potential (e.g. lipidperoxydation) of this irritant gas. Particle, asbestos fibre and irritant gas exposure may induce airway and pulmonary injury by the activation of AM and consecutive proinflammatory cytokine release.

Duthie SJ, Dobson VL. **Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro.** Z Ernahrungswiss 1999;38(1):28-34.
BACKGROUND & AIMS: Epidemiological studies suggest that antioxidant polyphenols in the human diet may protect against diseases such as cancer. In this study we investigated the cytoprotective potential of the flavonoids, quercetin, myricetin, kaempferol and rutin against oxidative DNA damage in human colonocytes in vitro. METHODS: Caco-2 cells, which display specialised enterocyte/colonocyte cell functions, were used as an in vitro model for human colonocytes. Hydrogen peroxide was employed as the oxidant. DNA damage (strand breakage, oxidised purines and oxidised pyrimidines) was determined using the alkaline single cell gel electrophoresis or comet assay. Cell growth and viability were measured. RESULTS: Hydrogen peroxide caused a dose-dependent increase in DNA strand breakage in human colonocytes, presumably via oxygen free radical generation. Quercetin and myricetin protected Caco-2 cells against oxidative attack. In addition, quercetin decreased hydrogen peroxide-mediated inhibition of growth. Neither rutin nor kaempferol was effective. However, quercetin, while inhibiting DNA strand breakage, did not alter the levels of oxidised bases following peroxide treatment. The antifungal agent ketoconazole, prevented quercetin cytoprotection in Caco-2 cells, indicating that P450-mediated metabolism may alter the efficacy of the flavonoids against oxidative DNA damage.

CONCLUSION: Flavonoids, particularly quercetin, the most abundant flavonoid in the human diet, are likely to be important in defending human colonocytes from oxidative attack.

Emonet-Piccardi N, Richard MJ, Ravanat JL, Signorini N, Cadet J, Beani JC. **Protective effects of antioxidants against UVA-induced DNA damage in human skin fibroblasts in culture.** Free Radic Res 1998;29(4):307-13.
Ultraviolet A radiation (UVA, 320-400 nm) is mutagenic and induces genomic damage to skin cells. N-acetyl-cysteine (NAC), selenium and zinc have been shown to have antioxidant properties and to exhibit protective effects against UVA cytotoxicity. The present work attempts to delineate the effect of these compounds on genomic integrity of human skin fibroblasts exposed to UVA radiation using the single cell gel electrophoresis (SCGE) or Comet assay. The cells were incubated with NAC (5 mM), sodium selenite (0.6 microM) or zinc chloride (100 microM). Then cells were embedded in low melting point agarose, and immediately submitted to UVA fluences ranging from 1 to 6J/cm2. In the Comet assay, the tail moment increased by 45% (1 J/cm2) to 89% (6J/cm2) in non-supplemented cells (p)<0.01). DNA damage was significantly prevented by NAC, Se and Zn, with a similar efficiency from 1 to 4J/cm2 ($p < 0.05$). For the highest UVA dose (6J/cm2), Se and Zn were more effective than NAC ($p < 0.01$).

Escher BI, Hunziker R, Schwarzenbach RP. **Kinetic model to describe the intrinsic uncoupling activity of substituted phenols in energy transducing membranes.** Environ Sci Technol 1999;33 (4):560-70.
BIOSIS COPYRIGHT: BIOL ABS. A new approach to understand the increased toxicity of uncouplers as compared to baseline toxicity (narcosis) is presented here. The overall uncoupling activity is quantitatively separated into the contribution of membrane concentration and speciation and intrinsic activity. This approach is a further step toward the development of improved Quantitative Structure-Activity Relationships (QSAR) and of toxicokinetic models used in risk assessment. The protonophoric uncoupling activity of seven nitro- and chlorophenols has been investigated as a function of pH and concentration using time-resolved spectroscopy on photosynthetic membranes. The experimental data are described by a kinetic model that includes a monomeric and a dimeric protonophoric shuttle mechanisms. Input parameters of the model are the experimental data for relaxation of the membrane potential, the biomembrane-water distribution constants of the phenol and phenoxide species, and the acidity constant of the phenol. Adjustable parameters are the translocation rate constants of all phenolic species and the heterodimer formation constant. These parameters constitute the intrinsic uncoupling activity. Hydrophobicity and acidity govern the partitioning of phenols into the membrane but appear not to be the sole determining factors for the intrinsic uncoupling activity of phenolic compounds. Additional factors include steric effects and charge distribution within the molecule.

Fisher JW, Mahle D, Abbas R. **A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol.** Toxicol Appl Pharmacol 1998;152(2):339-59.
Nine male and eight female healthy volunteers were exposed to 50 or 100 ppm trichloroethylene vapors for 4 h. Blood, urine, and exhaled breath samples were collected for development of a physiologically based pharmacokinetic (PBPK) model for trichloroethylene and its two major P450-mediated metabolites, trichloroacetic acid and free trichloroethanol. Blood and urine were analyzed for

trichloroethylene, chloral hydrate, free trichloroethanol and trichloroethanol glucuronide, and trichloroacetic acid. Plasma was analyzed for dichloroacetic acid. Trichloroethylene was also measured in exhaled breath samples. Trichloroethylene, free trichloroethanol, and trichloroacetic acid were found in blood samples of all volunteers and only trace amounts of dichloroacetic acid (4-12 ppb) were found in plasma samples from a few volunteers. Trichloroethanol glucuronide and trichloroacetic acid were found in urine of all volunteers. No chloral hydrate was detected in the volunteers. Gender-specific PBPK models were developed with fitted urinary rate constant values for each individual trichloroethylene exposure to describe urinary excretion of trichloroethanol glucuronide and trichloroacetic acid. Individual urinary excretion rate constants were necessary to account for the variability in the measured cumulative amount of metabolites excreted in the urine. However, the average amount of trichloroacetic acid and trichloroethanol glucuronide excreted in urine for each gender was predicted using mean urinary excretion rate constant values for each sex. A four-compartment physiological flow model was used for the metabolites (lung, liver, kidney, and body) and a six-compartment physiological flow model was used for trichloroethylene (lung, liver, kidney, fat, and slowly and rapidly perfused tissues). Metabolic capacity (Vmaxc) for oxidation of trichloroethylene was estimated to be 4 mg/kg/h in males and 5 mg/kg/h in females. Metabolized trichloroethylene was assumed to be converted to either free trichloroethanol (90%) or trichloroacetic acid (10%). Free trichloroethanol was glucuronidated forming trichloroethanol glucuronide or converted to trichloroacetic acid via back conversion of trichloroethanol to chloral (trichloroacetaldehyde). Trichloroethanol glucuronide and trichloroacetic acid were then excreted in urine. Gender-related pharmacokinetic differences in the uptake and metabolism of trichloroethylene were minor, but apparent. In general, the PBPK models for the male and female volunteers provided adequate predictions of the uptake of trichloroethylene and distribution of trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. The PBPK models for males and females consistently overpredicted exhaled breath concentrations of trichloroethylene immediately following the TCE exposure for a 2- to 4-h period. Further research is needed to better understand the biological determinants responsible for the observed variability in urinary excretion of trichloroethanol glucuronide and trichloroacetic acid and the metabolic pathway resulting in formation of dichloroacetic acid.

Folkes LK, Dennis MF, Stratford MR, Candeias LP, Wardman P. **Peroxidase-catalyzed effects of indole-3-acetic acid and analogues on lipid membranes, DNA, and mammalian cells in vitro.** Biochem Pharmacol 1999;57(4):375-82.

This study aimed to explore the mechanisms and molecular parameters which control the cytotoxicity of derivatives of indole-3-acetic acid (IAA) when oxidatively activated by horseradish peroxidase (HRP). Lipid peroxidation was measured in liposomes, damage to supercoiled plasmid DNA assessed by gel electrophoresis, free radical intermediates detected by EPR following spin trapping, binding of IAA-derived products demonstrated by 3H labelling, stable products measured by HPLC, and cytotoxicity in hamster fibroblasts measured by clonogenic survival. IAA, and nine analogues more easily oxidized by HRP, caused lipid peroxidation in liposomes, but not detectably in membranes of hamster fibroblasts, and were cytotoxic after HRP activation to varying degrees. Cytotoxicity was not correlated with activation rate. The hydrophilic vitamin E analogue, Trolox, inhibited cytotoxicity, whereas loading fibroblasts with vitamin E was ineffective, consistent with an oxidative mechanism in which radical precursors to damage are intercepted by Trolox in the aqueous phase. However, two known oxidation

products were nontoxic (the 3-carbinol and 3-aldehyde, both probably produced from 3-CH2OO* peroxyl radicals via the 3-CH*2 [skatolyl] radical following decarboxylation of the radical cation). The skatolyl radical from IAA was shown by EPR with spin trapping to react with DNA; electrophoresis showed binding to occur. Treatment of hamster fibroblasts with 5-3H-IAA/HRP resulted in intracellular bound 3H. Together with earlier results, the new data point to unknown electrophilic oxidation products, reactive towards intracellular targets, being involved in cytotoxicity of the IAA/HRP combination, rather than direct attack of free radicals, excited states, or membrane lipid peroxidation.

Freyberger A, Degen GH. **Inhibition of prostaglandin-H-synthase by o-phenylphenol and its metabolites.** Arch Toxicol 1998;72(10):637-44.
Chronic administration of o-phenylphenol (OPP) is known to induce urinary bladder tumours in the Fischer rat. The underlying toxic mechanism is poorly understood. Recently, arachidonic acid (ARA)-dependent, prostaglandin-H-synthase (PHS)-catalysed metabolic activation of the OPP metabolite phenylhydroquinone (PHQ) to a genotoxic species was suggested to be involved in OPP toxicity. To investigate this hypothesis in more detail, we have studied the effects of OPP and its metabolites on PHS. When microsomal PHS from ovine seminal vesicles (OSV) was used as enzyme source, both OPP, PHQ, and 2-phenyl-1,4-benzoquinone (PBQ) inhibited PHS-cyclooxygenase. The inhibitory potency was inversely related to the ARA concentration in the assay; at 7 microM ARA IC50-values were: 13 microM (OPP), 17 microM (PHQ), and 190 microM (PBQ). In cells cultured from OSV, which express high PHS activity, 40 microM OPP almost completely suppressed prostaglandin formation. Studies with microsomal PHS demonstrated that PHQ was an excellent substrate for PHS-peroxidase; both ARA and hydrogen peroxide supported oxidation to PBQ. OPP was only a poor substrate for PHS, but inhibited the ARA-mediated and to a lesser extent also the hydrogen peroxide-mediated in vitro oxidation of PHQ. Moreover, PHQ at up to moderately cytotoxic concentrations (50 microM) did not induce micronuclei in OSV cell cultures. Taken together, our findings do not provide evidence for an ARA-dependent, PHS-catalysed formation of genotoxic species from PHQ. Moreover, it seems to be questionable whether such activation can effectively occur in vivo, since OPP and PHQ turned out to be efficient cyclooxygenase inhibitors, and high levels of OPP and PHQ were found at least in the urine of OPP-treated rats. On the other hand, inhibition of the formation of cytoprotective prostaglandins in the urogenital tract may play a crucial role in OPP-induced bladder carcinogenesis.

Froetschl R, Chichmanov L, Kleeberg U, Hildebrandt AG, Roots I, Brockmoeller J. **Prediction of aryl hydrocarbon receptor-mediated enzyme induction of drugs and chemicals by mRNA quantification.** Chem Res Toxicol 1998;11(12):1447-52.
BIOSIS COPYRIGHT: BIOL ABS. Enzyme-specific testing for drug interactions by in vitro techniques has become a routine practice in drug development. With many drugs, enzyme induction has similar importance for the prediction of drug-drug interactions. We developed a method for recognizing enzyme induction mediated via the aryl hydrocarbon receptor. This type of induction may be clinically important since experimental data suggest a higher rate of toxification in induced subjects. Twenty-four drugs and environmental chemicals, selected as prototype inducers or being chemically related to known inducers, including HIV protease inhibitors nelfinavir, saquinavir, ritonavir, and indinavir, were tested for their potency to induce cytochrome P450 1A1 mRNA in human Hela cell cultures by a quantitative reverse transcriptase polymerase chain reaction. Known prototype inducers such as beta-naphthoflavone and 3-

methylcholanthrene exhibited the highest inducing potency quantified with an Imax value (maximal induction of cytochrome P450 1A1 mRNA synthesis) of 5.48 and 10.7ely. The enzyme-inducing efficacy of some compounds such as resveratrol (2.92lower (2.23-3.08imilar to benzimidazoles exhibited some extent of enzyme induction; e.g., Imax values were 0.86106 for omeprazole, lansoprazole, and losartan, respectively. To predict the clinical relevance of these inducing effects, the concentration at half-maximal induction IM was estimated; the plasma concentrations of these drug substances were within 1 order of magnitude of the IM values, upon usual dosage. In conclusion, cytochrome P450 1A1 enzyme induction by drugs is a common phenomenon, though there is a great range in the inducing efficacy. In vitro prediction of enzyme induction may be useful for explaining or foreseeing drug interactions, drug side effects, or toxicity by xenobiotics.

Frolik CA, Cain RL, Sato M, Harvey AK, Chandrasekhar S, Black EC, Tashjian AH Jr, Hock JM. **Comparison of recombinant human PTH(1-34) (LY333334) with a C-terminally substituted analog of human PTH-related protein(1-34) (RS-66271): in vitro activity and in vivo pharmacological effects in rats [see comments]**. J Bone Miner Res 1999;14(2):163-72.
Parathyroid hormone (PTH) and PTH-related protein (PTHrP) are believed to exert their biological actions through binding and activation of a common cell surface receptor. Recently, an analog of PTHrP (RS-66271), was described that demonstrated reduced binding affinity for the PTH/PTHrP receptor compared with bovine PTH(1-34) but retained equal biological activity. The present study investigated the receptor binding affinities of synthetic RS-66271 and recombinant human PTH(1-34) (LY333334) and compared their in vitro and in vivo pharmacological effects. RS-66271 had one hundredth the activity of PTH(1-34) in competing for the binding of [125I] [Nle8,18, Tyr34]human PTH(1-34) to the human PTH/PTHrP receptor stably expressed in a human kidney cell line. Despite this reduced binding affinity, RS-66271 had equivalent activity in increasing both cAMP production in osteoblast-like cells and bone resorption in neonatal mouse calvariae. However, RS-66271 was 7. 6-fold less active in stimulating inositol phosphate production. For in vivo studies, young, male Fisher rats received a daily subcutaneous dose of either 10 or 40 microg/kg of peptide for 1, 2, or 4 weeks. Volumetric bone mineral density and total bone mineral content of the proximal tibia were determined by peripheral quantitative computerized tomography. Trabecular and cortical bone of the distal femur were analyzed for calcium and dry weight. Lumbar vertebrae (L4-L6) were analyzed by histomorphometry. Trabecular and cortical bone mass showed a dose- and time-dependent increase in the treated animals compared with the controls. These increases were evident as early as 1 week after initiation of dosing. There were no consistent significant differences in the comparative effects of PTH(1-34) and RS-66271 on the measured bone parameters. In conclusion, despite the reduced binding affinity of RS-66271 for the PTH/PTHrP receptor compared with human PTH(1-34), both peptides displayed similar in vitro and in vivo pharmacological effects.

Fubini B. **Surface chemistry and quartz hazard.** Ann Occup Hyg 1998;42(8):521-30.
The variability of quartz hazard is related to the characteristics of particulate toxicants. Although these have the same chemical composition, they exist in various forms and surface states, each one eliciting different biological responses. On the basis of data from the literature, surface chemical properties are associated to the subsequent stages reported by Donaldson and Borm (1998) in the mechanistic model proposed for quartz carcinogenicity. Surface radicals and iron-derived reactive oxygen species (ROS)

are implicated in oxidative stress, considered to be the key event in the development of fibrosis and lung cancer. Other chemical functionalities related to cytotoxicity, however, modulate the overall pathogenicity by regulating transport and clearance. The chemical features deriving from the intrinsic characteristics of a silica dust--e.g. its origin--as well as those generated by external factors--e.g. contaminants, associated minerals--are discussed in relation to their possible role in the pathogenic mechanism.

Galey JB, Destree O, Dumats J, Pichaud P, Marche J, Genard S, Bracciolli G, Le Capitaine L, Plessix H, Brambilla L, et al. **Protection of U937 cells against oxidative injury by a novel series of iron chelators.** Free Radic Biol Med 1998;25(8):881-90.
A new series of iron chelators designed to protect tissues against iron-catalysed oxidative damage is described. These compounds are aminocarboxylate derivatives bearing pendant aromatic groups. They were designed to have a relatively low affinity for both ferrous and ferric iron and to be site-specifically oxidizable by hydrogen peroxide through intramolecular aromatic hydroxylation into species with strong iron binding capacity which do not catalyse hydroxyl radical formation. Thus, at the cellular level, oxidative injury is used to convert weak iron chelators into strong iron chelators in order to promote cell survival. The purpose of this local activation process is to minimise toxicity compared to strong iron chelators which may interfere with normal iron metabolism. Compounds within this series were evaluated in vitro in view of their capacity to undergo intramolecular hydroxylation and to protect cultured cells against oxidative injury. Results show that the intramolecular aromatic hydroxylation capacity is critically dependent upon the amino carboxylate chelating moieties and the substituents of the aromatic rings. Cell protection against oxidative injury is only observed with compounds possessing sufficient lipophilicity. The monohydroxylation product of N,N'-dibenzylethylenediamine N,N'-diacetic acid, protects cells against both H2O2 and tBuOOH toxicity with IC50's of 12 and 60 microM, respectively, in agreement with the oxidative activation concept. These results represent the first step toward the development of a new strategy to safe iron chelation for the prevention of oxidative damage.

Gibbs MA, Thummel KE, Shen DD, Kunze KL. **Inhibition of cytochrome P-450 3A (CYP3A) in human intestinal and liver microsomes: comparison of Ki values and impact of CYP3A5 expression.** Drug Metab Dispos 1999;27(2):180-7.
The purpose of this study was to compare the kinetics of intestinal and hepatic cytochrome P-450 3A (CYP3A) inhibition by using microsomal midazolam 1'-hydroxylation as a marker of enzyme activity. The effect of two antifungal agents commonly implicated in CYP3A drug-drug interactions was examined. Inhibition type and affinities were determined for human liver and intestinal microsomes screened for the presence or absence of CYP3A4 and CYP3A5, as well as for cDNA-expressed CYP3A4 and CYP3A5 microsomes. Ketoconazole and fluconazole were found to be noncompetitive inhibitors of both enzymes. Ketoconazole exhibited a Ki for cDNA-expressed CYP3A4 of 26. 7 +/- 1.71 nM, whereas the Ki for cDNA expressed CYP3A5 was 109 +/- 19.7 nM. Corresponding Ki values for fluconazole were 9.21 +/- 0.51 microM and 84.6 +/- 12.9 microM. For liver and intestinal microsomes that contained only CYP3A4, the average ketoconazole Ki was found to be 14.9 +/- 6.7 nM and 17.0 +/- 7.9 nM, respectively, whereas fluconazole yielded mean respective Ki values of 10.7 +/- 4.2 microM and 10.4 +/- 2.9 microM. Liver and intestinal microsomes that contained an equal or greater amount of CYP3A5, in addition to CYP3A4, were less susceptible to inhibition by both ketoconazole and

fluconazole. These findings suggest that there can be significant differences in the affinity of these two enzymes for inhibitors. This may further broaden interindividual variability with respect to the magnitude of in vivo drug-drug interactions. We also conclude that there is no significant difference in inhibition type and affinity of ketoconazole and fluconazole for hepatic versus intestinal CYP3A4.

Gichner T, Wagner ED, Plewa MJ. **Pentachlorophenol-mediated mutagenic synergy with aromatic amines in Salmonella typhimurium.** Mutat Res 1998;420(1-3):115-24.
Pentachlorophenol (PCP), a widely used pesticide, enhanced the mutagenic potency of plant- or mammalian-activated 2-aminofluorene (2AF) as well as the direct-acting mutagen 2-acetoxyacetylaminofluorene (2AAAF) when assayed with specific Salmonella typhimurium strains. With 2AF the mutagenic synergy was observed in strains YG1024, TA1538, and MP153. With 2AAAF the PCP-mediated synergy was observed with these strains and with strain TA98/1,8-DNP6. The synergy was dependent upon the presence of an activated N-acetoxy functional group and was only expressed at the hisD3052 allele and not at the hisG46 allele. Spectrophotometric analysis demonstrated that the rate of degradation of 2AAAF was reduced in the presence of PCP in phosphate buffer or with S. typhimurium cytosol and thus PCP may be affecting the stability of the N-acetoxy group of activated aromatic amines. Copyright 1998 Elsevier Science B.V.

Gisleskog PO, Hermann D, Hammarlund-Udenaes M, Karlsson MO. **A model for the turnover of dihydrotestosterone in the presence of the irreversible 5 alpha-reductase inhibitors GI198745 and finasteride.** Clin Pharmacol Ther 1998;64(6):636-47.
OBJECTIVE: To develop a pharmacokinetic-pharmacodynamic model that characterizes the conversion of testosterone to dihydrotestosterone (DHT) by 5 alpha-reductase types 1 and 2 and the irreversible inhibition of 5 alpha-reductase by finasteride, a 5 alpha-reductase type 2 inhibitor and by GI198745 (dutasteride), a potent and specific dual 5 alpha-reductase inhibitor. METHODS: Healthy men (n = 48) received doses of 0.1 to 40 mg GI198745 (n = 4 subjects per dose), 5 mg finasteride (n = 8), or placebo (n = 8) in a parallel-group study. Plasma concentrations of GI198745, finasteride, and DHT were measured frequently up to 8 weeks after dosing. Models were fitted with mixed-effects modeling with the NONMEM program. RESULTS: The pharmacodynamics were well described with a model that accounted for the rates of DHT formation and elimination, 5 alpha-reductase turnover, relative capacity of the 2 5 alpha-reductase isozymes, and the rates of irreversible inhibition of one (finasteride) or both (GI198745) types of 5 alpha-reductase. The model indicated that type 2 5 alpha-reductase contributed approximately 80% of plasma DHT. GI198745 was about 3-fold more potent than finasteride on 5 alpha-reductase type 2. Nearly full blockade of both isozymes was achieved at doses of 10 mg or more GI198745, although the potency of this agent on 5 alpha-reductase type 1 was less than on type 2. CONCLUSIONS: A physiologically based model for the turnover and irreversible inhibition of 5 alpha-reductase and for formation and elimination of DHT described the data well. This model helps explain differences in the rates of onset and offset of effect and offers a way to determine the relative potency of the irreversible 5 alpha-reductase inhibitors.

Godar DE. **UVA1 radiation triggers two different final apoptotic pathways.** J Invest Dermatol 1998;112(1):3-12.
Because ultraviolet-A1 (UVA1; 340-400 nm) radiation is used therapeutically, this in vitro study

addressed the question "how does it work?" To begin addressing this question, UVA1 radiation was first established to reduce the survival of transformed T and B lymphocytes in a linear dose-dependent manner using clonogenic reproductive assays, and that cell death occurs by apoptosis using transmission electron microscopy, Annexin V, and flow cytometry. The primary mechanism was determined to be immediate pre-programmed cell death, an apoptotic mechanism that does not require protein synthesis post-insult, by quantifying the apoptotic cells over time in the absence or presence of a translation inhibitor. To explore how UVA1 radiation induces immediate pre-programmed cell death apoptosis, reactive oxygen species and mitochondrial activity were altered during exposure using a variety of agents, while a specific fluorescent probe, 5,5',6,6'tetrachloro- 1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide, was used to examine mitochondrial transmembrane depolarization. To show that UVA1 mediates singlet-oxygen damage to the mitochondrial membranes, X-rays, UVB (290-320 nm), 8-methoxypsoralen and UVA, vitamin K3, anti-Fas antibody, and blocking antibody were the negative controls, while rose bengal or protoporphyrin IX with visible light were the positive controls. Cyclosporine A, which inhibits the mitochondrial megapore from opening, was used with singlet-oxygen and superoxide-anion generators to distinguish between the two final apoptotic pathways. The collective results show that UVA1 radiation primarily mediates singlet-oxygen damage triggering immediate pre-programmed cell death apoptosis (T < 20 min) by immediately opening the cyclosporine A-sensitive ("S" site) mitochondrial megapore, while superoxide anions initiate another cyclosporine A-insensitive ("P" site) final apoptotic pathway.

Goss PE, Baker MA, Carver JP, Dennis JW. **Inhibitors of carbohydrate processing: a new class of anticancer agents.** Clin Cancer Res 1995;1(9):935-44.
There is a need for anticancer agents with novel mechanisms of action. Recently identified molecular targets for new anticancer agents include inducers of cell differentiation, cell cycle arrest, and apoptosis, as well as signaling pathways for growth factors and cytokines. Another unexplored opportunity is presented by the ubiquitous intracellular glycoprotein glycosylation pathway. This complex process, concerned with the addition of sugars onto newly synthesized proteins, occurs in the lumen of the rough endoplasmic reticulum and in the Golgi. There are estimates of over 200 glycosyltransferase enzymes in this pathway, which results in considerable structural diversity of carbohydrates found on secreted and transmembrane glycoproteins. The specificity of glycosyltransferases for acceptors and sugar-nucleotide donors dictates linkage positions between sugars, anomeric configuration of linkages, and monosaccharide composition. Specific carbohydrate structures participate in cell-cell and cell-substratum interactions affecting processes such as lymphocyte trafficking, immune cell stimulation, embryogenesis, and cancer metastasis. Of the carbohydrate-processing inhibitors presently available, the alkaloid swainsonine, a Golgi alpha-mannosidase II inhibitor, is the first to have been selected for clinical testing based on its anticancer activity, p.o. availability, and low toxicity in mice. Herein, we review the rationale for targeting Golgi carbohydrate processing pathways in the treatment of cancer, and summarize the preclinical and clinical results with swainsonine. Prospects for the development of second generation inhibitors with improved specificity for Golgi-processing enzymes are discussed. Potential clinical applications of this new class of anticancer agents are emphasized.

Graether SP, Deluca CI, Baardsnes J, Hill GA, Davies PL, Jia Z. **Quantitative and qualitative analysis of type III antifreeze protein structure and function.** J Biol Chem 1999;274(17):11842-7.

Some cold water marine fishes avoid cellular damage because of freezing by expressing antifreeze proteins (AFPs) that bind to ice and inhibit its growth; one such protein is the globular type III AFP from eel pout. Despite several studies, the mechanism of ice binding remains unclear because of the difficulty in modeling the AFP-ice interaction. To further explore the mechanism, we have determined the x-ray crystallographic structure of 10 type III AFP mutants and combined that information with 7 previously determined structures to mainly analyze specific AFP-ice interactions such as hydrogen bonds. Quantitative assessment of binding was performed using a neural network with properties of the structure as input and predicted antifreeze activity as output. Using the cross-validation method, a correlation coefficient of 0.60 was obtained between measured and predicted activity, indicating successful learning and good predictive power. A large loss in the predictive power of the neural network occurred after properties related to the hydrophobic surface were left out, suggesting that van der Waal's interactions make a significant contribution to ice binding. By combining the analysis of the neural network with antifreeze activity and x-ray crystallographic structures of the mutants, we extend the existing ice-binding model to a two-step process: 1) probing of the surface for the correct ice-binding plane by hydrogen-bonding side chains and 2) attractive van der Waal's interactions between the other residues of the ice-binding surface and the ice, which increases the strength of the protein-ice interaction.

Greenberg MS, Burton GA, Fisher JW. **Physiologically based pharmacokinetic modeling of inhaled trichloroethylene and its oxidative metabolites in B6C3F1 mice.** Toxicol Appl Pharmacol 1999;154 (3):264-78.

A physiologically based pharmacokinetic (PBPK) model for inhaled trichloroethylene (TCE) was developed for B6C3F1 mice. Submodels described four P450-mediated metabolites of TCE, which included chloral hydrate (CH), free and glucuronide-bound trichloroethanol (TCOH-f and TCOH-b), trichloroacetic acid (TCA), and dichloroacetic acid (DCA). Inhalation time course studies were carried out for calibration of the model by exposing mice to TCE vapor concentrations of either 100 or 600 ppm for 4 h. At several time points, mice were euthanized and blood, liver, kidney, lung, and fat were collected and analyzed for TCE and its oxidative metabolites. Peak blood TCE concentrations were 0.86 and 7.32 microgram/mL, respectively, in mice exposed to 100 and 600 ppm TCE. The model overpredicted the mixed venous blood and tissue concentrations of TCE for mice of both exposure groups. Fractional absorption of inhaled TCE was proposed to explain the discrepancy between the model predictions and the TCE blood time course data. When fractional absorption (53%) of inhaled TCE was incorporated into the model, a comprehensive description of the uptake, distribution, and clearance of TCE in the blood was obtained. Fractional uptake of inhaled TCE was further verified by collecting TCE in exhaled breath following a 4-h constant concentration exposure to TCE and validation was provided by testing the model against TCE blood concentrations from an independent data set. The submodels adequately simulated the distribution and clearance kinetics of CH and TCOH-f in blood and the lungs, TCOH-b in the blood, and TCA and DCA, which were respectively detected for up to 43 and 14 h postexposure in blood and livers of mice exposed to 600 ppm TCE. This is the first extensive tissue time course study of the major metabolites of TCE following an inhalation exposure to TCE and the PBPK model predictions were in good general agreement with the observed kinetics of the oxidative metabolites formed in mice exposed to TCE concentrations of 100 and 600 ppm.

Gres MC, Julian B, Bourrie M, Meunier V, Fabre G. **Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line**. Pharm Res 1998 May;15:726-33.
IPA COPYRIGHT: ASHP The relationship between oral drug absorption in humans and the apparent permeability coefficients of drugs in 2 human intestinal epithelial cell lines, the parental Caco-2 cell line and its clone, the TC-7 cell line, was studied using 20 drugs exhibiting large differences in chemical structure, molecular weight, transport mechanisms, and percentage of absorption in humans. The results showed that both the Caco-2 and TC-7 cell lines are valuable tools for predicting the passive transport of drugs in humans. Some parameters helped to demonstrate that the TC-7 cell line would be a more valuable tool for investigating transport characteristics than the Caco-2 cell line.

Harada J, Sugimoto M. **Inhibitors of interleukin-1 beta-converting enzyme-family proteases (caspases) prevent apoptosis without affecting decreased cellular ability to reduce 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide in cerebellar granule neurons.** Brain Res 1998;793(1-2):231-43.
We assessed the possible role of interleukin-1beta-converting enzyme-family proteases (caspases) in apoptosis in cultured rat cerebellar granule neurons. CPP32 (caspase-3)-like protease activity was augmented by low KCl treatment, preceding neuronal cell death. Agents such as brain-derived neurotrophic factor (BDNF), dibutylyl cAMP, NMDA, actinomycin D, S-adenosyl-L-methionine, and spermine prevented apoptosis. For various neuroprotective agents, the degree of apoptosis prevention correlated with the prevention of the activation of CPP32-like protease. Furthermore, Z-Asp-2, 6-dichlorobenzoyloxy-methylketone (Z-Asp-CH2-DCB), Boc-Asp-fluoromethylketone (Boc-Asp-FMK), and Z-Val-Ala-Asp-fluoromethylketone (Z-VAD-FMK), which are inhibitors of caspases, also prevented apoptosis. In contrast to many other neuroprotective agents, these inhibitors of caspases showed little effect on the decrease of cellular 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) reduction activity after low KCl treatment. The neurons rescued by these inhibitors of caspases during low KCl treatment were in a hypoenergic state in their ATP levels and vulnerable to subsequent treatment with medium containing high KCl or glutamate which induce an influx of Ca2+, but which are less toxic to normal neurons. These results suggest that caspase(s) are involved in the apoptosis of cerebellar granule neurons and that several agents protect neurons from death by blocking the activation of the protease(s). Although several caspase inhibitors examined in this study protect neurons from apoptosis, rescued neurons are vulnerable to subsequent stimuli that induce necrotic cell death. Copyright 1998 Elsevier Science B.V.

Harrison CA, Raftery MJ, Walsh J, Alewood P, Iismaa SE, Thliveris S, Geczy CL. **Oxidation regulates the inflammatory properties of the murine S100 protein S100A8.** J Biol Chem 1999;274(13):8561-9.
The myeloid cell-derived calcium-binding murine protein, S100A8, is secreted to act as a chemotactic factor at picomolar concentrations, stimulating recruitment of myeloid cells to inflammatory sites. S100A8 may be exposed to oxygen metabolites, particularly hypochlorite, the major oxidant generated by activated neutrophils at inflammatory sites. Here we show that hypochlorite oxidizes the single Cys residue (Cys41) of S100A8. Electrospray mass spectrometry and SDS-polyacrylamide gel electrophoresis analysis indicated that low concentrations of hypochlorite (40 microM) converted 70-

80% of S100A8 to the disulfide-linked homodimer. The mass was 20,707 Da, 92 Da more than expected, indicating additional oxidation of susceptible amino acids (possibly methionine). Phorbol 12-myristate 13-acetate activation of differentiated HL-60 granulocytic cells generated an oxidative burst that was sufficient to efficiently oxidize exogenous S100A8 within 10 min, and results implicate involvement of the myeloperoxidase system. Moreover, disulfide-linked dimer was identified in lung lavage fluid of mice with endotoxin-induced pulmonary injury. S100A8 dimer was inactive in chemotaxis and failed to recruit leukocytes in vivo. Positive chemotactic activity of recombinant Ala41S100A8 indicated that Cys41 was not essential for function and suggested that covalent dimerization may structurally modify accessibility of the chemotactic hinge domain. Disulfide-dependent dimerization may be a physiologically significant regulatory mechanism controlling S100A8-provoked leukocyte recruitment.

Harrison KL, Jukes R, Cooper DP, Shuker DE. **Detection of concomitant formation of O6-carboxymethyl- and O6-methyl-2'-deoxyguanosine in DNA exposed to nitrosated glycine derivatives using a combined immunoaffinity/HPLC method.** Chem Res Toxicol 1999;12(1):106-11.
A previous observation that an N-nitroso-N-carboxymethyl derivative reacts with DNA to give both O6-carboxymethyl-2'-deoxyguanosine (O6-CMdGuo) and O6-methyl-2'-deoxyguanosine (O6-MedGuo) [Shuker, D. E. G., and Margison, G. P. (1997) Cancer Res. 57, 366-369] has been confirmed using a range of nitrosated glycine derivatives [N-acetyl-N'-nitroso-N'-prolylglycine (APNG), azaserine (AS), and potassium diazoacetate (KDA)]. In addition, mesyloxyacetic acid (MAA) was also found to give both O6-adducts in DNA. O6-CMdGuo and O6-MedGuo were assessed in enzymatic hydrolysates of treated calf thymus DNA using a combined immunoaffinity/HPLC/fluorescence procedure. The ratio of O6-CMdGuo to O6-MedGuo varied somewhat between the different compounds with APNG giving the most methylation (O6-CM:O6-Me ratio of 10) and AS the least (39), with KDA and MAA giving intermediate amounts (16 and 18, respectively). The formation of O6-MedGuo by the four compounds probably arises through decarboxylation at various stages in the decomposition pathways, but the exact mechanisms remain to be clarified. The formation of O6-MedGuo from reactions of nitrosated glycine derivatives with DNA in vitro may explain the frequent detection of this adduct in human gastrointestinal DNA, as nitrosation of dietary glycine may occur. O6-CMdGuo is likely to be a useful biomarker of this pathway in vivo and has been detected in human tissues.

Hinshaw DB, Lodhi IJ, Hurley LL, Atkins KB, Dabrowska MI. **Activation of poly [ADP-Ribose] polymerase in endothelial cells and keratinocytes: role in an in vitro model of sulfur mustard-mediated vesication.** Toxicol Appl Pharmacol 1999;156(1):17-29.
Although endothelial cells and keratinocytes appear to be the primary cellular targets of sulfur mustard (SM), the role of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) in SM-induced vesication has not been clearly defined. PARP is thought to play a crucial role in DNA repair mechanisms following exposure to alkylating agents like SM. Using a combination of fluorescence microscopy and biochemical assays, we tested the hypothesis that SM causes activation of PARP in endothelial cells and keratinocytes with subsequent loss of nicotinamide adenine dinucleotide (NAD) and depletion of adenosine triphosphate (ATP) levels. To determine if PARP activation accounts for SM-induced vesication, keratinocyte adherence and permeability of endothelial monolayers were measured as in vitro correlates of vesication. As early as 2 to 3 h after exposure to SM concentrations as low as 250 microM,

dramatic changes were induced in keratinocyte morphology and microfilament architecture. Exposure to 500 microM SM induced a fourfold increase in PARP activity in endothelial cells, and a two- to threefold increase in keratinocytes. SM induced a dose-related loss of NAD+ in both endothelial cells and keratinocytes. ATP levels fell to approximately 50% of control levels in response to SM concentrations >/=500 microM. SM concentrations >/=250 microM significantly reduced keratinocyte adherence as early as 3 h after exposure. Endothelial monolayer permeability increased substantially with concentrations of SM >250 microM. These observations support the hypothesis that the pathogenic events necessary for SM-induced vesication (i.e., capillary leak and loss of keratinocyte adherence) at higher vesicating doses of SM (>/=500 microM) may depend on NAD loss with PARP activation and subsequent ATP-dependent effects on microfilament architecture. Vesication developing as a result of exposure to lower concentrations of SM presumably occurs by mechanisms that do not depend on loss of cellular ATP (e.g., apoptosis and direct SM-mediated damage to integrins and the basement membrane). Copyright 1999 Academic Press.

Hirano N, Haruki M, Morikawa M, Kanaya S. **Stabilization of ribonuclease HI from Thermus thermophilus HB8 by the spontaneous formation of an intramolecular disulfide bond.** Biochemistry 1998;37(36):12640-8.
To identify factors that contribute to the thermal stability of ribonuclease HI (RNase HI) from Thermus thermophilus HB8, protein variants with a series of carboxyl-terminal truncations and Cys --> Ala mutations were constructed, and their thermal denaturations were analyzed by CD. The results indicate that Cys41 and Cys149 contribute to the protein stability, probably through the formation of a disulfide bond. Peptide mapping analysis for the mutant protein with only two cysteine residues, at positions 41 and 149, indicated that this disulfide bond is partially formed in a protein purified from Escherichia coli in the absence of a reducing reagent but is fully formed in a thermally denatured protein. These results suggest that the thermal stability of T. thermophilus RNase HI, determined in the absence of a reducing reagent, reflects that of an oxidized form of the protein. Comparison of the thermal stabilities and the enzymatic activities of the wild-type and truncated proteins, determined in the presence and absence of a reducing reagent, indicates that the formation of this disulfide bond increases the thermal stability of the protein by 6-7 degreesC in Tm and approximately 3 kcal/mol in DeltaG without seriously affecting the enzymatic activity. Since T. thermophilus RNase HI is present in a reducing environment in cells, this disulfide bond probably is not formed in vivo but is spontaneously formed in vitro in the absence of a reducing reagent.

Horii I. **Advantages of toxicokinetics in new drug development.** Toxicol Lett 1998;102-103:657-64.

Ishaque A, Al-Rubeai M. **Use of intracellular pH and annexin-V flow cytometric assays to monitor apoptosis and its suppression by bcl-2 over-expression in hybridoma cell culture.** J Immunol Methods 1998;221(1-2):43-57.
Accurate identification and quantitation of apoptosis is essential for developing efficient strategies for optimisation of culture viability and productivity in cell lines of industrial significance. We have examined the possibility of using carboxy-seminaphthorhodafluor-1-acetoxymethylester (carboxy SNARF-1-AM), a pH sensitive fluoroprobe and FITC-labelled annexin V (AV), a probe specific to phosphatidylserine exposed on the surface of apoptotic cells, to monitor apoptosis and to determine the

relationship between intracellular pH (pHi), apoptosis and cell cycle in hybridoma cells. Temporal changes in the distribution of proliferative capacity (S phase), metabolic activity (pHi), and cell death population dynamics were effectively and reliably determined using flow cytometry. Intracellular acidification was shown to precede the occurrence of apoptosis during batch culture and after treatment with campothecin, staurosporine and under adverse bioreactor conditions such as glutamine deprivation and oxygen deficiency. These results showed that the decrease in pHi can be used as an indicator of cellular deterioration and cell death. AV in combination with propidium iodide permitted the identification of viable, transient apoptotic and necrotic cells in heterogeneous cultures of control (PEF) cells. Hybridoma cells over-expressing bcl-2 were protected from intracellular acidification and phosphatidylserine exposure, which was associated with the suppression of apoptosis in these cells. A decrease in pHi was apparent even before the accumulation of the normally acidic G1 phase and the development of a sub-G1 region, characteristic of apoptotic cell behaviour. The pHi assay can therefore be used as a tool to predict future cell culture performance. reserved.

Jarnberg J, Johanson G. **Physiologically based modeling of 1,2,4-trimethylbenzene inhalation toxicokinetics.** Toxicol Appl Pharmacol 1999;155(3):203-14.
A physiologically based toxicokinetic model was developed for inhalation exposure of 1,2,4-trimethylbenzene (TMB) in man. The model consists of six compartments for TMB and one compartment for the metabolite 3,4-dimethylhippuric acid (DMHA). Based on previous experimental findings from human exposures to TMB, liver metabolism was divided in two pathways, one of the first order and one of the Michaelis-Menten type. Muscle tissue was split in two compartments to account for working and resting muscle tissues during bicycle exercise. The model was used to investigate how various factors influence potential biomarkers of exposure, i.e., TMB in blood and exhaled air and DMHA in urine. Increasing the work load from rest to moderate exercise (100 W) more than doubled all biomarker levels end of shift. The effect on next morning levels was even more pronounced, illustrated by a fivefold increase in the DMHA excretion rate. Simulations of five daily 8-h exposures suggest that biomarker levels end of shift remain fairly constant whereas the levels prior to shift increase gradually during the week. This suggests that end of shift levels reflect the exposure of the same day whereas levels Friday morning reflect exposure during the entire working week. Simulations with randomly generated exposures show that the variability due to fluctuating exposure is lower next morning than end of shift. End of shift exhalation rate of TMB is more sensitive to fluctuation than TMB in venous blood and DMHA in urine. Biomarker levels for 25 ppm exposure at different sampling times are given. Copyright 1999 Academic Press.

Kanamaru K, Wang R, Su W, Crawford NM. **Ser-534 in the hinge 1 region of Arabidopsis nitrate reductase is conditionally required for binding of 14-3-3 proteins and in vitro inhibition.** J Biol Chem 1999;274(7):4160-5.
14-3-3 proteins bind to the hinge 1 region of nitrate reductase (NR) and inhibit its activity. To determine which residues of NR are required for 14-3-3-inhibitory interactions, wild-type and mutant forms of Arabidopsis NR were examined in the yeast two-hybrid system and in vitro inhibition assays. NR fragments with or without hinge 1 were introduced into yeast with one of seven Arabidopsis 14-3-3 isoforms (called GF14s). NR fragments (residues 1-562 or 487-562) containing hinge 1 interacted with all GF-14s tested; an NR fragment (residues 1-487) lacking hinge 1 did not. GF14 binding to NR

fragments was dependent on Ser-534, since Asp or Ala substitutions at this site blocked the interaction. Revertants with second site substitutions restoring interaction between GF14omega and the Ala- or Asp-substituted NR fragments were identified. One isolate had a Lys to Glu substitution at position 531, which is in hinge 1, and six isolates had Ile to Leu or Phe substitutions at 561 in the heme binding region. Double mutant forms of holo-NR (S534D plus K531E, I561F, or I561L) were constructed and found to be partially inhibited by protein extracts from Arabidopsis containing 14-3-3 proteins. Wild-type NR is phosphorylated and inhibited by these extracts, but S534D single mutant forms are not. These results show that inhibitory NR/14-3-3 interactions are dependent on Ser-534 but only in the context of the wild-type sequence, since substitutions at second sites render 14-3-3 binding and in vitro NR inhibition independent of Ser-534.

Katsir G, Parola AH. **Enhanced proliferation caused by a low frequency weak magnetic field in chick embryo fibroblasts is suppressed by radical scavengers.** Biochem Biophys Res Commun 1998;252(3):753-6.
Sinusoidal varying magnetic fields (SVMF) were reported by us to enhance the proliferation of chick embryo fibroblasts (CEF). The mechanism through which SVMF affects biological systems is still enigmatic. While the SVMF examined by us (50, 60, and 100 Hz/0.06-0.7 mT) were all below kT, they may have the potential of altering chemical processes in which excited radicals are involved. We tested this hypothesis by subjecting CEF to radical scavengers during exposure to a magnetic field of 100 Hz and 0.7 mT for 24 h. Cell proliferation was evaluated by MTT colorimetric assay. In the presence of catalase, superoxide dismutase, or vitamin E, the SVMF enhanced cell proliferation was reduced by 79, 67, and 82%, respectively. The addition of exogenous radical scavengers to the cells during the exposure to magnetic field significantly suppressed the enhancement in cell proliferation caused by the field.

Khamiss O, Lery X, Belal MH, Badawy HA, Gianotti J, Abol-Ela SM. **Effects of some insecticides on the division of a Spodoptera littoralis cell line and on the replication of Sl baculovirus (NPV).** Appl Entomol Zool 1998;33(3):349-55.
 The impact of several insecticides has been studied on a Spodoptera littoralis cell line and their effects on the replication of S. littoralis nucleopolyhedrovirus (Sl NPV). Four chemical insecticides, Chloropyrifos, Fenitrothion, Cypermethrin and Carbaryl which belong to three different groups of pesticide, organophosphorus, pyrethroid, and carbamate, respectively, were used. The results demonstrate an increase of more than 25% in the multiplication of Sl cells when treated with Chloropyrifos dilutions compared with untreated cells. The most destructive pesticide was Cypermethrin, which caused 84% inhibition of cell development (0.4concentration of 10-3 mug/ml in preliminary tests. In the presence of both Sl NPV and sublethal doses of these insecticides, the TCID50 values revealed that the Cypermethrin (10-8 mug/ml) was the most toxic (ID50 was 6.48s was the least toxic (ID50 9.29 of 0.731 mug/ml. The PFU values confirm the results that the same concentration of Chloropyrifos (0.731 mug/ml) was the least toxic (PFU 6.41 (4.47e different insecticides the PFU values ranged between 2.88 for Chloropyrifos at a concentration of 10-3 mug/ml and 9.76 1012 for Carbaryl at 10-5 mug/ml. A comparison with the results obtained using Sl NPV without chemicals (the PFU 3.5ml) indicates an increase in the production of virus and titer, and a synergistic effect of the chemicals. These results confirm the effect of these insecticides in vivo. As the Cypermethrin may have a potent effect on

the exchange of ions through the cell membrane in vitro and in vivo, it could be partially explain the impact of the insecticides and their mode of action.

Lelong IH, Rebel G. **In vitro taurine uptake into cell culture influenced by using media with or without CO2.** J Pharmacol Toxicol Methods 1998;39(4):211-20.
Buffers used to incubate cells for pharmacological or toxicological studies are usually of very simple composition, far from the composition of biological fluids or cell culture media. Comparative studies on taurine uptake levels by cultured cells show that a new CO2-Independent Medium (CIM) is suitable for incubating cells in place of the Krebs-Ringer buffer (KR) usually used. Basal uptake level of taurine was lower for cells incubated in CIM or in other culture media when compared to those incubated whether in KR or in other "physiological buffers." Isoproterenol depressed similarly the taurine uptake in cells incubated in CIM or KR. The same uptake modulation by beta-alanine, GES, GABA, or HEPES was observed for cells incubated in CIM or KR. C6 cells growth in CIM was dependent on the starting cell density when classically vented T-flasks were used, growth being notably reduced at low density. In tightly closed flasks cells grew in CIM similarly to control cultures maintained in M199 medium or DMEM.

Lelong IH, Rebel G. **pH drift of "physiological buffers" and culture media used for cell incubation during in vitro studies.** J Pharmacol Toxicol Methods 1998;39(4):203-10.
In pharmacological or toxicological studies performed at room atmosphere comparison of various media used for cell incubation revealed discrepancies among results due to pH instability when these media contain bicarbonate. With the classically used protocols, a relatively fast and notable rise of the pH of such media has been observed, and values higher than 8.5 could be reached after 1 h of incubation. A less important rise in pH was also observed for media containing low amounts of sodium bicarbonate, e. g., Hank's formula-derived media. Because Hepes-buffered media or media with abnormal osmolarity cannot always be used for such studies, our choice of media is limited.

Lin JH. **Applications and limitations of interspecies scaling and in vitro extrapolation in pharmacokinetics.** Drug Metab Dispos 1998;26(12):1202-12.
The search for new drugs is an extremely time-consuming and costly endeavor. Much of the time and cost are expended on generating data that support the efficacy and safety profiles of the drug. Because of ethical constraints, relevant pharmacological and toxicological assessments must be made in laboratory animals and in in vitro systems before human testing can begin. In support of the efficacy and safety evaluation during drug development, two fundamental challenges facing industrial drug metabolism scientists are (1) how to "scale-up" the pharmacokinetic data from animals to humans and (2) how to extrapolate the in vitro data to the in vivo situation. This review examines the applications and limitations of interspecies scaling and in vitro extrapolation in pharmacokinetics.

Lin JH, Lu AY. **Inhibition and induction of cytochrome P450 and the clinical implications.** Clin Pharmacokinet 1998;35(5):361-90.
The cytochrome P450s (CYPs) constitute a superfamily of isoforms that play an important role in the oxidative metabolism of drugs. Each CYP isoform possesses a characteristic broad spectrum of catalytic activities of substrates. Whenever 2 or more drugs are administered concurrently, the possibility of drug

interactions exists. The ability of a single CYP to metabolise multiple substrates is responsible for a large number of documented drug interactions associated with CYP inhibition. In addition, drug interactions can also occur as a result of the induction of several human CYPs following long term drug treatment. The mechanisms of CYP inhibition can be divided into 3 categories: (a) reversible inhibition; (b) quasi-irreversible inhibition; and (c) irreversible inhibition. In mechanistic terms, reversible interactions arise as a result of competition at the CYP active site and probably involve only the first step of the CYP catalytic cycle. On the other hand, drugs that act during and subsequent to the oxygen transfer step are generally irreversible or quasi-irreversible inhibitors. Irreversible and quasi-irreversible inhibition require at least one cycle of the CYP catalytic process. Because human liver samples and recombinant human CYPs are now readily available, in vitro systems have been used as screening tools to predict the potential for in vivo drug interaction. Although it is easy to determine in vitro metabolic drug interactions, the proper interpretation and extrapolation of in vitro interaction data to in vivo situations require a good understanding of pharmacokinetic principles. From the viewpoint of drug therapy, to avoid potential drug-drug interactions, it is desirable to develop a new drug candidate that is not a potent CYP inhibitor or inducer and the metabolism of which is not readily inhibited by other drugs. In reality, drug interaction by mutual inhibition between drugs is almost inevitable, because CYP-mediated metabolism represents a major route of elimination of many drugs, which can compete for the same CYP enzyme. The clinical significance of a metabolic drug interaction depends on the magnitude of the change in the concentration of active species (parent drug and/or active metabolites) at the site of pharmacological action and the therapeutic index of the drug. The smaller the difference between toxic and effective concentration, the greater the likelihood that a drug interaction will have serious clinical consequences. Thus, careful evaluation of potential drug interactions of a new drug candidate during the early stage of drug development is essential.

Lipscomb JC, Fisher JW, Confer PD, Byczkowski JZ. **In vitro to in vivo extrapolation for trichloroethylene metabolism in humans.** Toxicol Appl Pharmacol 1998;152(2):376-87.
The use of in vitro systems in the assessment of xenobiotic metabolism has distinct advantages and disadvantages. While isolated hepatocytes and microsomes prepared from human liver may be used to generate data for comparisons among species and in vitro systems, such comparisons are generally performed on the basis of microsomal protein or million (viable) hepatocytes. Recently, in vitro data have been investigated for their value as quantitative predictors of in vivo metabolic capacity. Because of the existence of large amounts of trichloroethylene (TRI) data in the human, we have examined the metabolism of TRI as a case study in the development of a method to compare metabolism across species using in vitro systems and for extrapolation of metabolic rates from in vitro to in vivo. TRI is well metabolized by human hepatocytes in culture with a K(m) of 266 +/- 202 ppm (mean +/- SD) in headspace and a Vmax of 16.1 +/- 12.9 nmol/h/10(6) viable hepatocytes. We determined that human liver contains approximately 116 x 10(6) hepatocytes and 20.8 mg microsomal protein/g, based on DNA recovery and glucose-6-phosphatase activity, respectively. Thus, the microsomal protein content of hepatocytes is 179 micrograms microsomal protein/10(6) isolated hepatocytes. The microsomal apparent Vmax value of 1589 pmol/min/mg microsomal protein extrapolates to 17.07 nmol/h/10(6) hepatocytes. The combination of protein recovery and metabolic rate predicted a Vmax of approximately 1400 nmol/h/g human liver, which, when extrapolated and incorporated into an existing physiologically based pharmacokinetic (PBPK) model for TRI, slightly underpredicted TRI metabolism in the intact human.

The quantitation, extrapolation, and inclusion of extrahepatic and cytochrome P450 (CYP)-independent TRI metabolism may increase the predictive value of this approach.

Lizano C, Sanz S, Luque J, Pinilla M. **In vitro study of alcohol dehydrogenase and acetaldehyde dehydrogenase encapsulated into human erythrocytes by an electroporation procedure.** Biochim Biophys Acta 1998;1425(2):328-36.
BIOSIS COPYRIGHT: BIOL ABS. The optimal conditions for electroporated/resealed loading of alcohol dehydrogenase (ADH) and/or acetaldehyde dehydrogenase (ALDH) into human erythrocytes were established prior to the study, with the following characteristics: 300 V, 1 ms pulse time, eight pulses every 15 min and 1 h resealing at 37~C. High encapsulation yield and carrier cell recoveries were achieved. Cell volumes increase while hemoglobin contents decrease; in consequence a decrease in cell hemoglobin concentration was observed. A lower hypotonic resistance of loading erythrocytes (throughout osmotic fragility curves) and unaltered oxygen transport capability (as given by oxygen equilibrium curves) were observed. The stability against time (up to 168 h-7 days) of encapsulated individual enzymes, either ADH- or ALDH-red blood cells (RBCs), was studied at 4~C and 37~C, in comparison with that of free enzyme solutions. Both enzymes were released from carrier RBCs to the incubation medium. The stability of carrier RBCs was studied under similar conditions. Non-significant variations in hematological parameters were observed. However, the hemoglobin derivative forms showed modifications. The continuous degradation of ethanol by ADH-RBCs and coencapsulated ADH- and ALDH-RBCs, as a function of time (up to 70 h) suggests the use of these carrier RBCs as agents for complete metabolization of ethanol. The mentioned properties bare the possibility of using ADH and ALDH as carrier systems in in vivo situations.

Martin FL, Mclean AE. **Comparison of paracetamol-induced hepatotoxicity in the rat in vivo with progression of cell injury in vitro in rat liver slices.** Drug Chem Toxicol 1998;21(4):477-94.
The flux in rat hepatic ratio of adenosine triphosphate levels to adenosine diphosphate levels (ATP/ADP) during the onset and progression of paracetamol-induced cell injury both in vivo and in vitro were investigated and compared. Leakage of lactate dehydrogenase (LDH) and potassium (K+), and mg water/mg dry weight quantified cell injury. ATP and ADP levels were determined using the luciferin-luciferase bioluminescence assay. For in vitro studies, liver slices obtained from phenobarbitone-induced rats were exposed to 10 mM paracetamol for 120 min (T0-T120) and, then incubated without paracetamol up to a further 240 min (T120-T360). For in vivo studies, groups of four phenobarbitone-induced rats received i.p. injections of 800 mg/kg paracetamol. ATP/ADP ratios fall upon exposure to paracetamol both in vitro and in vivo. However, unlike the in vitro situation where the fall in ATP/ADP ratios precedes and accompanies the progression of cell injury, the in vivo fall in ATP/ADP ratios is shown to occur as cell injury measurements begin to recover to control levels. However, despite these differences classic paracetamol-induced centrilobular necrosis is observed to occur both in vitro and in vivo. This study demonstrates that the liver slice model is a simple and useful technique to investigate the underlying mechanisms of paracetamol-induced cell injury.

Mason H, Wilson K. **Biological monitoring: the role of toxicokinetics and physiologically based pharmacokinetic modeling.** Am Ind Hyg Assoc J 1999;60(2):237-42.
This short review outlines the contribution of modeling techniques, particularly physiologically based

pharmacokinetic (PBPK) modeling, in promulgating biological monitoring as a practical tool for the occupational health professional. The impact of modeling techniques is discussed in helping to establish the relevant biomarkers to measure, the appropriate time of sampling, and the relationship between atmospheric exposure limits and concentration of biological analyte. Of particular interest is the use of "population" PBPK techniques. These can explore the influence of physiological differences between workers or of particular susceptible subgroups (e.g., pregnant women, breast-feeding mothers, and infants) on the relationship between atmospheric exposure levels and biomarker concentration. Such techniques will become more widely used as biological monitoring guidance values (e.g., biological exposure indices, biological tolerance values) are increasingly established by various international professional and regulatory bodies.

Motoyama K, Karl IE, Flye MW, Osborne DF, Hotchkiss RS. **Effect of Ca2+ agonists in the perfused liver: determination via laser scanning confocal microscopy.** Am J Physiol 1999;276(2 Pt 2):575-85.
Ca2+ is a critical intracellular second messenger, but few studies have examined Ca2+ signaling in whole organs. The amplitude and frequency of Ca2+ oscillations encode important cellular information. Using laser scanning confocal microscopy in the indo 1 acetoxymethyl ester dye-loaded rat liver, we investigated the effect of various Ca2+ agonists that act at distinct mechanistic sites on Ca2+ signaling. Perfusion with suprathreshold doses of arginine vasopressin (AVP) (2-20 nM) caused a single Ca2+ wave that originated in the pericentral vein region and spread centrifugally to the periportal area. Lower doses of AVP (0.2-2 nM) caused multiple Ca2+ waves and Ca2+ oscillations. Perfusion with ATP (1. 4-17.5 microM) caused rapid transient elevations in intracellular free Ca2+ concentration ([Ca2+]i) occurring in isolated hepatocytes or groups of hepatocytes throughout the lobule and were of shorter duration than those due to AVP. Also in contrast to AVP, there was no specific anatomic location within the hepatic lobule that was more susceptible to ATP. Thapsigargin and cyclopiazonic acid did not cause a Ca2+ wave but rather produced a uniform and fairly simultaneous increase in [Ca2+]i in all hepatocytes in the lobule. Perfusion with 14 microM ryanodine produced a single transient spike in [Ca2+]i in a small number (<2%) of hepatocytes. Dantrolene, an inhibitor of Ca2+ release, reduced the increased [Ca2+]i occurring after AVP. Insight into the mechanism of action of these Ca2+-active compounds on Ca2+ signaling in the intact liver is provided.

Murray FJ. **A comparative review of the pharmacokinetics of boric acid in rodents and humans.** Biol Trace Elem Res 1998;66(1-3):331-41.
The pharmacokinetics of boric acid (BA) have been studied in animals and humans. Orally administered BA is readily and completely absorbed in rats, rabbits, and humans, as well as other animal species. In animals and humans, absorbed BA appears to be rapidly distributed throughout the body water via passive diffusion. Following administration of BA, the ratio of blood: soft tissue concentrations of boron (B) is approx 1.0 in rats and humans; in contrast, concentrations of B in bone exceed those in blood by a factor of approx 4 in both rats and humans. In rats, adipose tissue concentrations of B are only 20% of the levels found in blood and soft tissues; however, human data on adipose tissue levels are not available. BA does not appear to be metabolized in either animals or humans owing to the excessive energy required to break the B-O bond. BA has an affinity for cis-hydroxy groups, and it has been hypothesized to elicit its biological activity through this mechanism. The elimination kinetics of BA also appear to be similar for rodents and humans. BA is eliminated unchanged in the urine. The kinetics of

elimination were evaluated in human volunteers given BA orally or intravenously; the half-life for elimination was essentially the same (approx 21 h) by either route of exposure. In rats, blood and tissue levels of B reached steady-state after 3-4 d of oral administration of BA; assuming first-order kinetics, a half-life of 14-19 h may be calculated. The lack of metabolism of BA eliminates metabolic clearance as a potential source of interspecies variation. Accordingly, in the absence of differences in metabolic clearance, renal clearance is expected to be the major determinant of interspecies variation in pharmacokinetics. Because glomerular filtration rates are slightly higher in rats than in humans, the slight difference in half-lives may be readily explained. The most sensitive toxicity end point for BA appears to be developmental toxicity in rats, with a No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) of 55 and 76 mg BA/kg/d, respectively. Mean blood B levels in pregnant rats on gestation day 20 in the pivotal developmental toxicity study were reported to be 1.27 and 1.53 mcg B/g at the NOAEL and LOAEL, respectively. Blood B concentrations in humans are well below these levels. Average blood B levels in the most heavily exposed worker population at a borate mine was 0.24 mcg B/mL, and the estimated daily occupational exposure was equivalent to 160 mg BA/d. Blood B levels in the general population generally range from 0.03 to 0.09 mcg B/mL. These blood B values indicate an ample margin of safety for humans. In summary, the pharmacokinetics of BA in humans and rodents are remarkably similar, and interspecies differences in pharmacokinetics appear to be minimal.

Nestorov IA, Aarons LJ, Arundel PA, Rowland M. **Lumping of whole-body physiologically based pharmacokinetic models**. J Pharmacokinet Biopharm 1998 Feb;26:21-46.
IPA COPYRIGHT: ASHP The lumping process, a common pragmatic approach aimed at the reduction of whole body physiologically based pharmacokinetic (PBPK) modelling, is described and examined from a system theory point of view as a basis for formulating the principles and standard procedures that lumping should follow; theoretical considerations are illustrated with an example taken from PBPK modeling.

Nestorov IA, Aarons LJ, Rowland M. **Physiologically based pharmacokinetic modeling of a homologous series of barbiturates in the rat: sensitivity analysis**. J Pharmacokinet Biopharm 1997 Aug;25:413-47.
IPA COPYRIGHT: ASHP Two sensitivity analysis approaches, perturbation analysis and direct sensitivity function analysis, were applied to a whole-body physiologically based pharmacokinetic model of the distribution of a homologous series of nine 5-n-alkyl-5-ethyl barbituric acid derivatives after intravenous bolus administration in rats in order to rank the model parameters involved according to their impact on the model outputs and to study the changes in the sensitivity induced by increases in the lipophilicity of the homologs. It was noted that the computationally simple perturbation analysis can be used when only orientation about the sensitivity of a system is required. On the other hand, direct sensitivity analysis provides comprehensive information but requires sophisticated simulation programs, extensive numerical and modeling expertise, and a large number of calculations.

Nisoli E, Clementi E, Tonello C, Sciorati C, Briscini L, Carruba MO. **Effects of nitric oxide on proliferation and differentiation of rat brown adipocytes in primary cultures.** Br J Pharmacol 1998;125(4):888-94.

1. In the present work, we study the effect of NO on the proliferation and differentiation of brown fat cells in primary cultures. 2. Brown fat precursor cells isolated from rat brown adipose tissue were cultured for 8 days until confluence and treated daily with the NO donating agents, S-nitroso-acetyl penicillamine (SNAP) or S-nitroso-L-glutathione (GSNO). Both agents (300 microM) decreased cell proliferation approximately 8 fold on day 8. The inhibitory effect of NO was unlikely to be due to cytotoxicity since (i) cells never completely lost their proliferation capacity even after 8 days of exposure to repeated additions of SNAP or GSNO, and (ii) the inhibitory effect was reversible after removal of the media containing NO donors. 3. Daily treatment with nitric oxide synthase inhibitors, such as NG-nitro-L-arginine methyl ester (L-NAME, 300 microM), led to the stimulation of cell proliferation by 44+/-5%, n=3, suggesting that NO, endogenously produced in brown adipocytes, may be involved in modulating cell growth. 4. Daily treatment with both SNAP or GSNO induced significant mitochondriogenesis, measured as the mitochondrial conversion of 3-[4,5-dimethylthiazol-2-yl-]-2,5-diphenyl tetrazolium bromide (MTT) to formazan, whilst daily treatment with L-NAME was without effect. 5. The inhibition of cell proliferation by NO donors was accompanied by the expression of two genes coding for peroxisome proliferator activated receptor-gamma and uncoupling protein-1, which are upregulated during differentiation. 6. Increasing cyclic GMP in cells by 8-bromo-cyclic GMP (100-1000 microM) did not reproduce the observed NO effects on either cell number or gene expression. On the other hand, chronic treatment with the inhibitor of the NO-stimulated guanylyl cyclase, 1H-[1,2,4] oxadiazole[4,3-a]quinoxalin-1-one (ODQ), reduced the expression of peroxisome proliferator activated receptor-gamma and uncoupling protein-1.

Normand-Sdiqui N, Akhtar S. **Oligonucleotide delivery: uptake of rat transferrin receptor antibody (OX-26) conjugates into an in vitro immortalized cell line model of the blood-brain barrier**. Int J Pharm 1998 Mar 18;163:63-71.
IPA COPYRIGHT: ASHP To examine the ability of OX-26, a monoclonal antibody that recognizes the rat transferrin receptor, to function as an effective carrier for the delivery of oligonucleotides to the brain, the in vitro uptake of OX-26/oligonucleotide conjugates in the RBE4 cell line, an immortalized rat brain endothelial cell line that is a model of the blood/brain barrier was studied; the effect of momensin on the uptake of the OX-26/oligonucleotide conjugates in the RBE4 cell line was also examined. The uptake of OX-26/oligonucleotide conjugates in the RBE4 cell line was 2-fold greater than that of free oligonucleotides, and the uptake mechanism was consistent with transferrin receptor-mediated endocytosis. Treatment of the RBE4 cells with momensin further increased the intracellular accumulation of the OX-26/oligonucleotide conjugates, suggesting that uptake of the conjugates may involve the trans-Golgi network.

Oliver RE, Heatherington AC, Jones AF, Rowland M. **Physiologically based pharmacokinetic model incorporating dispersion principles to describe solute distribution in the perfused rat hindlimb preparation**. J Pharmacokinet Biopharm 1997 Aug;25:389-412.
IPA COPYRIGHT: ASHP A physiologically based pharmacokinetic model, incorporating dispersion principles, that describes the distribution of inert reference markers, including sucrose, urea, and water, drugs such as 5-n-alkyl-5-ethyl barbituratic acid derivatives, and 2 intravascular markers, RBC and albumin, in an in situ perfused rat hindlimb preparation is discussed; the drugs and inert and intravascular markers were radiolabeled.

Ornaghi P, Ballario P, Lena AM, Gonzalez A, Filetici P. **The bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4.** J Mol Biol 1999;287(1):1-7.
Whereas the histone acetyltransferase activity of yeast Gcn5p has been widely studied, its structural interactions with the histones and the role of the carboxy-terminal bromodomain are still unclear. Using a glutathione S-transferase pull down assay we show that Gcn5p binds the amino-terminal tails of histones H3 and H4, but not H2A and H2B. The deletion of bromodomain abolishes this interaction and bromodomain alone is able to interact with the H3 and H4 N termini. The amino acid residues of the H4 N terminus involved in the binding with Gcn5p have been studied by site-directed mutagenesis. The substitution of amino acid residues R19 or R23 of the H4 N terminus with a glutamine (Q) abolishes the interaction with Gcn5p and the bromodomain. These residues differ from those known to be acetylated or to be involved in binding the SIR proteins. This evidence and the known dispensability of the bromodomain for Gcn5p acetyltransferase activity suggest a new structural role for the highly evolutionary conserved bromodomain. Copyright 1998 Academic Press.

Osman NE, Westrom B, Karlsson B. **Serosal but not mucosal endotoxin exposure increases intestinal permeability in vitro in the rat.** Scand J Gastroenterol 1998;33(11):1170-4.
BACKGROUND: Microbial endotoxins are normally present in the gut, usually without apparent harmful effects, whereas systemically administered endotoxin impairs the mucosal barrier function. Our aim was to investigate whether in vitro exposure to bacterial lipopolysaccharide (LPS) could affect the intestinal barrier properties of the rat small intestine. METHODS: Small-intestinal segments from rats were mounted in Ussing diffusion chambers, and the mucosal to serosal permeation of the marker molecules bovine serum albumin (BSA) and 51Cr-ethylenediaminetetraacetic acid (EDTA) was measured after addition of LPS to the mucosal or serosal side. RESULTS: Mucosal exposure to LPS (0.01, 0.05, 0.25 mg/ml) had no effects on the permeation of BSA and 51Cr-EDTA, whereas when added to the serosal side at 0.05 or 0.25 mg/ml, LPS increased the marker permeation. CONCLUSION: Serosal LPS exposure in vitro increased the intestinal permeability to the different-sized markers, whereas mucosal LPS did not, indicating that the mechanisms leading to intestinal barrier impairment can be initiated in the intestinal wall itself.

Pahlavani MA, Harris MD. **Effect of in vitro generation of oxygen free radicals on T cell function in young and old rats.** Free Radic Biol Med 1998;25(8):903-13.
T cells from young (6 months) and old (24 months) male Fischer 344 rats were isolated and exposed to three different oxidative stress conditions: (a) reactive oxygen species generated by xanthine-xanthine oxidase (X/XO), (b) hydrogen peroxide (H2O2), and (c) hyperthermia (43 degrees C for 1 h). After oxidative stress treatment, the induction of proliferation and IL-2 production by concanavalin A (Con A) was measured. Exposure of T cells to X/XO or H2O2 resulted in suppression of proliferation and IL-2 expression, and the suppressive effect was more pronounced in T cells from young rats than in T cells from old rats. Similarly, hyperthermia caused inhibition of proliferation and IL-2 expression in T cells from young and old rats. Addition of antioxidant to cultured cells only slightly attenuated the effects of X/XO and H2O2 on T cell function; however, antioxidant had no effect on heat shock-mediated inhibition of proliferation in young or old rats. Because IL-2 plays a crucial role in T cell proliferation and because the transcription factor NFAT (nuclear factor of activated T cell) plays a major role in the

regulation of IL-2 transcription, the induction of NFAT as well as NF-KB and AP-1 DNA binding activities in nuclear extracts of the X/XO-treated and untreated control cells was measured using a gel shift assay. The ability of nuclear extracts to bind NFAT or NF-KB oligonucleotide decreased in the X/XO-treated cells from young and old rats compared to the untreated controls. Therefore, these data imply that reactive oxygen species generated by the X/XO system alter the distal step of mitogen-mediated signal transduction, i.e., transcription factors that regulate IL-2 transcription.

Paolini M, Pozzetti L, Perocco P, Mazzullo M, Cantelli-Forti G. **Molecular non-genetic biomarkers of effect related to methyl thiophanate cocarcinogenesis: organ- and sex-specific cytochrome P450 induction in the rat.** Cancer Lett 1999;135(2):203-13.
We used selective biochemical markers of effect to evaluate some non-genotoxic cocarcinogenic properties of methyl thiophanate (MTH) associated with cytochrome P450 (CYP) changes. Several CYP-dependent reactions were monitored in the liver, kidney and lung microsomes of male and female Sprague-Dawley rats treated (i.p.) with a single (285 or 570 mg/kg body weight) or repeated (daily 285 or 570 mg/kg body weight for three consecutive days) doses of this pesticide. No significant changes in absolute or relative liver, kidney and lung weights were observed after MTH injection. Highly specific substrates were used as probes of different isoforms, such as CYP1A1, 1A2, 2B1, 2E1 and 3A. A complex pattern of CYP induction, including organ- and sex-related differences, was observed, particularly in the liver (CYP3A, 2B1), kidney (CYP1A1, 2E1) and lung (CYP3A, 1A1). In the liver, an increase up to 29-fold in the 2B1-like activity, probed by the O-dealkylation of pentoxyresorufin, was observed at lower dose in both sexes, and the induction of CYP 1A2-mediated methoxyresorufin O-demethylase activity (up to 3.6-fold) was recorded at the higher dose in males. In the kidney, the O-deethylation of ethoxyresorufin (CYP1A1-linked) was increased up to 28.2-fold and the CYP2E1-dependent p-nitrophenol hydroxylases were enhanced up to 6.3-fold in females receiving higher multiple MTH administration. In the lung, the CYP3A-associated activity was the most induced oxidases, as exemplified by the marked increase in the O-demethylation of aminopyrine (up to 3.6-fold) in males. A weak, although significant, reduction of CYP2B1-linked oxidases was also observed in repeated treatment in the kidney (males) and lung (females). These results suggest that the induction of CYP-catalyzed drug metabolism by prolonged exposure to MTH may result in accelerated metabolism of coadministered drugs with important implications for their disposition Together with an alteration of endogenous metabolism, the adverse effects associated with CYP changes such as toxicity/cotoxicity, cocarcinogenicity and promotion may also have clinical consequences.

Pathiratne A, George SG. **Toxicity of malathion to nile tilapia, Oreochromis niloticus and modulation by other environmental contaminants.** Aquatic Toxicol 1998;43(4):261-71.
BIOSIS COPYRIGHT: BIOL ABS. Deliberate or accidental contamination of ponds by widely utilized organophophorous (OP) insecticides such as malathion is a potential problem for aquaculture in tropical countries. The aim of the study was to investigate potential synergistic or protective effects of common environmental pollutants on malathion toxicity in the Nile tilapia (Oreochromis niloticus) and by correlation of acute toxicity (LC50) studies with biochemical parameters, identify potential enzyme systems involved in malathion toxicity. Tilapia were very sensitive to malathion (96h LC50 2ppm) and in vitro data indicated that malaoxon, formed by oxidation of malathion, was the effective toxicant. Exposure of fish to an environmentally relevant dose of the insecticide synergist and CYP inhibitor,

piperonyl butoxide (PBO) markedly reduced both the sublethal and the acute toxicity of malathion by 2-fold. Correlation of toxicity data with inducer effects and biochemical analyses failed to provide any evidence for CYP1-, CYP2B- or CYP3A-mediated malathion activation or detoxication in this species, thus the effect of PBO could not be attributed to inhibition of these enzymes. Whilst interspecies comparisons implicate hepatic theta class GST and non-specific carboxylesterase in malathion detoxication there was no evidence for alterations in malathion toxicity to tilapia by inducers of these enzymes. Treatment of fish with concentrations of a prototypical polyaromatic hydrocarbon, or cadmium. exceeding those producing effects in field situations, did not alter malathion toxicity indicating a lack of interaction of other common classes of environmental pollutants with OP toxicity.

Pedrajas JR, Gavilanes F, Lopez-Barea J, Peinado J. **Incubation of superoxide dismutase with malondialdehyde and 4-hydroxy-2-nonenal forms new active isoforms and adducts. An evaluation of xenobiotics in fish.** Chem Biol Interact 1998;116(1-2):1-17.
The effects in fish (Sparus aurata) of dieldrin, previously reported to be an inducer of peroxisomal enzymes (Pedrajas et al., Comp. Biochem. Physiol. 115C (1996) 125-131), were compared with those of clofibrate. Although dieldrin provoked the more severe peroxisomal changes, both compounds induced oxidative stress as detected by the increased levels of microsomal thiobarbituric acid reactive substances; however the malondialdehyde (MDA) content, determined after HPLC separation of the MDA-TBA complex, was not significantly altered. These results suggest that, besides MDA, other aldehydes were formed in xenobiotic-injected fish, leading us to assess the oxidative effects of such xenobiotics by following changes in superoxide dismutase (SOD) pattern. New active SOD isoforms were detected by isoelectrofocusing in the light mitochondrial (LMF) and cytosolic (CF) fractions. Most of the new SOD bands could be reproduced in vitro by incubation of fish liver cell-free extracts with MDA. To clarify the effects of aldehydes, Cu,Zn- and Mn-SOD isoforms were purified and amino acid analysis was carried out. The new bands found in LMF and CF fractions were reproduced in vitro after incubation of pure SODs with MDA and 4-hydroxy-2-nonenal (HNE), the new SOD bands formed being coincident with the loss of Lys or His residues. Lysine residues were preferentially derivatized after treatment of Cu,Zn-SOD with MDA, but in Mn-SOD the lysine residues were modified only after treatment with MDA, while the histidine residues were modified only by HNE. No change of SOD activity was detected after MDA or HNE exposure, although at the higher aldehyde concentrations used protein aggregates were formed. Therefore, the appearance of new active SOD bands, after isoelectrofocusing separation, can be proposed as a biomarker of oxidative stress.

Penta K, Varner JA, Liaw L, Hidai C, Schatzman R, Quertermous T. **Del1 induces integrin signaling and angiogenesis by ligation of alphaVbeta3.** J Biol Chem 1999;274(16):11101-9.
Del1 is a novel extracellular matrix protein encoding three Notch-like epidermal growth factor repeats, an RGD motif, and two discoidin domains. Del1 is expressed in an endothelial cell-restricted pattern during early development. In studies reported here, recombinant baculovirus Del1 protein was shown to promote alphavbeta3-dependent endothelial cell attachment and migration. Attachment of endothelial cells to Del1 was associated with clustering of alphavbeta3, the formation of focal complexes, and recruitment of talin and vinculin into these complexes. These events were shown to be associated with phosphorylation of proteins in the focal complexes, including the time-dependent phosphorylation of p125(FAK), MAPK, and Shc. When recombinant Del1 was evaluated in an in ovo chick chorioallantoic

membrane assay, it was found to have potent angiogenic activity. This angiogenic activity was inhibited by a monoclonal antibody directed against alphavbeta3, and an RAD mutant Del1 protein was inactive. Thus Del1 provides a unique autocrine angiogenic pathway for the embryonic endothelium, and this function is mediated in part by productive ligation of integrin alphavbeta3.

Philip PA, Ali-Sadat S, Doehmer J, Kocarek T, Akhtar A, Lu H, Chan KK. **Use of V79 cells with stably transfected cytochrome P450 cDNAs in studying the metabolism and effects of cytotoxic drugs.** Cancer Chemother Pharmacol 1999;43(1):59-67.
PURPOSE: Studying the metabolism of cytotoxic drugs has become increasingly necessary to predict clinically significant drug-drug interactions and to understand the basis of interindividual variations in the pharmacokinetics of anticancer agents. The aim of this study was to determine the feasibility of using V79 Chinese hamster fibroblasts, which are stably transfected with cytochrome P450 (CYP) cDNAs, to study the metabolism of cytotoxic drugs in vitro. METHODS: The 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell survival after incubation with drugs. Gas chromatography/mass spectroscopy was used for the quantitation of metabolites of cyclophosphamide and ifosfamide in culture medium. The coculture technique was used to study the generation of cytotoxic metabolites in culture medium. RESULTS: After treatment with either cyclophosphamide or ifosfamide (100 microM to 1 mM) cytotoxicity was demonstrated in only cytochrome CYP2B1- and cytochrome CYP3A4-expressing cells. Treatment of parental nontransfected cells that were cocultured with CYP-expressing cells with cyclophosphamide resulted in increased sensitivity to this drug. All active and inactive metabolites of cyclophosphamide and ifosfamide were detected in the culture medium. Cyclophosphamide-induced cytotoxicity in CYP2B1- and CYP3A4-expressing cells was abrogated by metyrapone and midazolam/ troleandomycin, respectively. Paclitaxel showed greater cytotoxicity against parental V79 cells than against the CYP2BI-, 2E1-, or 3A4-expressing cells, which was also influenced by cotreatment with CYP inhibitors. CONCLUSIONS: Stable expression of CYP cDNAs by V79 cells provided an in vitro system to study cytotoxic drug metabolism. Cell viability and metabolite assays were used to determine the differential metabolism and effects in different CYP-transfected cell lines treated with cytotoxic drugs. The potential use of this V79 cell expression system is in studying enzymes involved in the metabolism of cytotoxic drugs, especially early in drug development. In addition, this system may be used to determine drug interactions that may influence the outcome of therapy in patients with cancer.

Renauld AE, Melancon MJ, Sordillo LM. **Identification of in vitro cytochrome P450 modulators to detect induction by prototype inducers in the mallard duckling (Anas platyrhynchos).** Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1999;122(2):273-81.
Seven modulators of mammalian monooxygenase activity were screened for their ability to selectively stimulate or inhibit in vitro monooxygenase activities of hepatic microsomes from mallard ducklings treated with phenobarbital, beta-naphthoflavone, 3,3',4,4',5-pentachlorobiphenyl or vehicle. Microsomes were assayed fluorometrically for four monooxygenases: benzyloxy-, ethoxy-, methoxy-, and pentoxyresorufin-O-dealkylase, in combination with each of the seven modulators. Four combinations: alpha-naphthoflavone and 2-methylbenzimidazole with benzyloxyresorufin, and Proadifen with methoxy- and ethoxyresorufin, respectively, were evaluated further. beta-Naphthoflavone-treated groups were clearly distinguished from the corn oil vehicle control group by all of the assays and by the effects

of the modulators in three of the four assay/modulator combinations. Enzyme activities of the phenobarbital and saline groups were statistically similar (P > or = 0.05) when assayed without modulator added, but each assay/modulator combination distinguished between these groups. The PCB-treated group was distinguished from the corn oil vehicle control group only for BROD activity, with or without the presence of modulator. Graphing of per cent modulation of BROD activity versus initial BROD activity provided the clearest distinction between all of the study groups. Identification of these selective in vitro modulators may improve detection and measurement of low level cytochrome P450 induction in avian species. Also, both the monooxygenase activities induced and the impacts of the modulators indicated differences between mammalian and avian cytochromes P450.

Rodgers EH, Grant MH. **The effect of the flavonoids, quercetin, myricetin and epicatechin on the growth and enzyme activities of MCF7 human breast cancer cells.** Chem Biol Interact 1998;116 (3):213-28.
Humans ingest about 1 g of flavonoids daily in their diet, and they are increasingly being associated with cytoprotective antitumour properties. The mechanism(s) responsible for these effects have not yet been elucidated but may involve interaction with xenobiotic metabolising enzymes to alter the metabolic activation of potential carcinogens. We have investigated the effect of the flavonoids, quercetin (Q), myricetin (M) and epicatechin (E) on the growth, morphology and enzyme activities of MCF7 human breast cancer cells. Of the three flavonoids studied only Q caused a decrease in cell protein content and decreased the reduction of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium). It also inhibited protein, DNA and RNA synthesis to the greatest extent. Q and M increased intracellular reduced glutathione (GSH) content, and Q altered the morphology of the cells after 24 h exposure to 25 microM. E and Q inhibited the O-deethylation of ethoxyresorufin (EROD) catalysed by cytochrome P450 CYPIA. In contrast, M increased the EROD reaction 2-fold. Q increased the activity of DT-diaphorase, NADPH cytochrome c reductase and glutathione reductase, while E increased only NADPH cytochrome c reductase activity. The effects on enzyme activities in vitro suggest that there is not only the potential for flavonoids to alter metabolic activation of carcinogens but also of therapeutically administered drugs in vivo. We are at present investigating the synergy between anti-cancer drugs and flavonoids in terms of anti-tumour efficacy.

Rodriguez Cruz MS, Gonzalez Alonso I, Sanchez-Navarro A, Sayalero Marinero ML. **In vitro study of the interaction between quinolones and polyvalent cations.** Pharm Acta Helv 1999;73(5):237-45.
The aim of the present study was to evaluate the influence of aluminium and iron on the in vitro dissolution kinetics of ciprofloxacin and ofloxacin as well as the usefulness of this type of in vitro data to predict modifications in in vivo absorption processes as a consequence of different factors, such as the widely documented in vivo interaction between quinolones and cations. Fitting of experimental data to different theoretical in vitro dissolution profiles was performed by non-linear regression methods and the statistical moments were calculated from raw experimental data. Analysis of residuals applied to dissolution curves as well as statistical comparison of the estimated parameters were carried out to evaluate the in vitro interaction. The results reveal significative modifications of the dissolution profiles of these quinolones as a consequence of the presence of cations, especially for Fe2+ which decreases 34.7% the maximum amount dissolved for ciprofloxacin and 29.1% for ofloxacin. Al3+ also produces a decrease of the total amount of quinolone dissolved although less relevant than Fe2+. Analysis of

residuals proved to be the best statistical method to evaluate differences between whole dissolution profiles, at least under the experimental conditions used.

Roser M, Fischer D, Kissel T. **Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats.** Eur J Pharm Biopharm 1998;46(3):255-63.
The surface charges on biodegradable albumin nanoparticles were introduced by covalent coupling different primary amines to examine their influence on phagocytosis by macrophages under in vitro conditions. Albumin particles with a zeta potential close to zero showed a reduced phagocytic uptake in comparison with charged particles, especially nanoparticles with a positive zeta potential. The phagocytic uptake in the present study was examined using an established cell culture model based on primary mouse peritoneal macrophages and a human hematopoietic monocytic cell line (U-937) treated with phorbol-12-myristic-13-acetate to induce cell differentiation. The influence of opsonins on in vitro phagocytosis experiments was characterized using carriers pre-treated with human serum. In the presence of human serum the phagocytic activity of U-937 cells was found to be similar to primary mouse macrophages without serum. In contrast to peritoneal macrophages, U-937 cells showed no phagocytic activity in the absence of serum. In particular, only the C3b- complement deposition on the particle surface seems to promote the phagocytic process. The in vivo distribution of albumin carriers in rats was investigated using magnetic resonance imaging (MRI). No differences in blood circulation times and organ accumulation between different nanoparticle preparations with positive, neutral and negative surface charges could be observed in rats, suggesting that the in vivo fate of albumin nanoparticles is significantly influenced by factors not reflected in the in vitro cell culture models.

Sandoval-Chacon M, Thompson JH, Zhang XJ, Liu X, Mannick EE, Sadowska-Krowicka H, Charbonnet RM, Clark DA, Miller MJ. **Antiinflammatory actions of cat's claw: the role of NF-kappaB.** Aliment Pharmacol Ther 1998;12(12):1279-89.
BACKGROUND: Uncaria tomentosa is a vine commonly known as cat's claw or 'una de gato' (UG) and is used in traditional Peruvian medicine for the treatment of a wide range of health problems, particularly digestive complaints and arthritis. PURPOSE: The aim of this study was to determine the proposed anti-inflammatory properties of cat's claw. Specifically: (i) does a bark extract of cat's claw protect against oxidant-induced stress in vitro, and (ii) to determine if UG modifies transcriptionally regulated events. METHODS: Cell death was determined in two cell lines, RAW 264.7 and HT29 in response to peroxynitrite (PN, 300 microM). Gene expression of inducible nitric oxide synthase (iNOS) in HT29 cells, direct effects on nitric oxide and peroxynitrite levels, and activation of NF-kappaB in RAW 264.7 cells as influenced by UG were assessed. Chronic intestinal inflammation was induced in rats with indomethacin (7.5 mg/kg), with UG administered orally in the drinking water (5 mg/mL). RESULTS: The administration of UG (100 microg/mL) attenuated ($P < 0.05$) peroxynitrite-induced apoptosis in HT29 (epithelial) and RAW 264.7 cells (macrophage). Cat's claw inhibited lipopolysaccharide-induced iNOS gene expression, nitrite formation, cell death and inhibited the activation of NF-kappaB. Cat's claw markedly attenuated indomethacin-enteritis as evident by reduced myeloperoxidase activity, morphometric damage and liver metallothionein expression. CONCLUSIONS: Cat's claw protects cells against oxidative stress and negated the activation of NF-

kappaB. These studies provide a mechanistic evidence for the widely held belief that cat's claw is an effective anti-inflammatory agent.

Schick CS, Haller C. **Comparative cytotoxicity of ionic and non-ionic radiocontrast agents on MDCK cell monolayers in vitro.** Nephrol Dial Transplant 1999;14(2):342-7.
BACKGROUND: Intravascular radiocontrast agents may cause acute renal failure, particularly in patients with pre-existing renal insufficiency. Direct cytotoxic effects of radiocontrast agents on renal tubular cells may contribute to the pathogenesis of radiocontrast-induced nephropathy. METHODS: We analysed the cytotoxicity of the ionic radiocontrast agents diatrizoate (monomeric) and ioxaglate (dimeric), as well as of the non-ionic radiocontrast agents iohexol (monomeric) and iodixanol (dimeric) on the renal epithelial Madin Darby Canine Kidney (MDCK) cell line grown on permeable supports. The toxicity assays assessed cell viability, transmonolayer resistance and inulin permeability between the apical and basal cell culture compartment. In addition, the distribution of the tight-junction-associated membrane proteins ZO-1 and occludin was analysed using immunofluorescence microscopy. RESULTS: In all assays the high osmolal ionic compound diatrizoate had significant cytotoxic effects that included the partial redistribution of the tight-junction-associated membrane proteins into a cytoplasmic compartment. To a lesser extent this redistribution also occurred with the dimeric ionic compound ioxaglate, but not with the non-ionic radiocontrast agents. With regards to cell viability, transmonolayer resistance and inulin permeability the radiocontrast agents with reduced osmolality were significantly less toxic than diatrizoate, independent of their ionic strength. CONCLUSIONS: Physicochemical factors contribute to the cytotoxicity of radiocontrast agents in vitro. The redistribution of tight-junction-associated membrane proteins by the ionic radiocontrast agents corresponds with the loss of the barrier function of the epithelial cell monolayer, which is a major pathophysiological mechanism in acute renal failure. The radiocontrast agents with reduced osmolality are less cytotoxic than diatrizoate, independent of their ionicity. Hyperosmolality appears to be a more important determinant of the cytotoxicity of diatrizoate than ionic strength.

Schoeffner DJ, Warren DA, Muralidara S, Bruckner JV, Simmons JE. **Organ weights and fat volume in rats as a function of strain and age.** J Toxicol Environ Health 1999;56(7):449-62.
The Fischer 344 (F344) rat and the Sprague-Dawley (SD) rat are used commonly to evaluate potential adverse health effects resulting from environmental exposure to chemicals. They are also the most common rat strain/stock used in physiologically based pharmacokinetic (PBPK) modeling. Accurate characterization of model input parameters will improve the usefulness of PBPK model predictions. Thus, organ (i.e., liver, kidneys, spleen, stomach, small intestine, large intestine, heart, lungs, brain) weights and body fat were measured in male SD rats of different ages (4 to 40 wk) and in young (9 to 10 wk) and old (22 to 23 mo) male F344 rats. Comparison of age-matched (9 to 10 wk) F344 and SD rats revealed that the SD rats weighed significantly more and had significantly higher absolute organ weights. These significant differences usually disappeared when organ weights were expressed as a percentage of body weight (relative organ weight). Percent body fat was significantly lower in the age-matched SD rats (6.48%) than in their F344 counterparts (8.67%). As expected, both body weight and absolute organ weights were significantly higher in old than in young F344 rats. However, these differences were largely reversed when relative organ weights were considered, with most relative organ weights significantly lower in the old F344 rats. Body fat as a percentage of body weight was 14.02% in

the old F344 rats. When SD rats of various ages were examined, relative organ weights declined between the ages of 4 and 14 wk. In contrast, significant differences in percent body fat were not detected among the SD rats of different ages and weights examined in this study (4 to 40 wk, approximately 75 to approximately 450 g). In summary, values for physiological input parameters are provided that should prove useful in development and implementation of more accurate PBPK models.

Sherwood RJ, Sinclair GC. **New PBPK model applied to old occupational exposure to benzene.** Am Ind Hyg Assoc J 1999;60(2):259-65.
An intensive program of benzene monitoring using new techniques was undertaken in Western Europe in the late 1960s and early 1970s. Significant exposure was found in the transport of benzene and gasoline, particularly during the loading of barges, and during the loading and operation of sea-going vessels. The ceiling threshold limit value of 25 ppm recommended at that time generated problems in assessing exposure, so alternative criteria were proposed. During that period some shore-based exposures were reported, and their significance was discussed in several articles. The information gained at that time is reexamined by physiologically based pharmacokinetic (PBPK) modeling and is used to help validate an improved PBPK model, which is described and tested on results from experimental exposure in a companion article. The old field data, comprising five specific studies, confirm the relevance of modeling to assessment of occupational exposure, and demonstrate its value for interpretation of field data, which is seldom as complete, systematic, or accurate as that obtained in experimental work. The model suggests that metabolism of benzene in humans may not be restricted to the liver. Sites and processes of metabolism merit further investigation.

Shim JY, Boone PF, Richard AM. **Theoretical study of the SNV reaction of trichloroethylene (TCE) and CH3S- as a model for glutathione conjugation of TCE.** Chem Res Toxicol 1999;12(4):308-16.
Trichloroethylene (TCE), a major environmental pollutant, is activated to mutagenic and nephrotoxic intermediates through a glutathione (GSH) conjugation pathway. Three product isomers of GSH-TCE conjugation, having potentially different toxicities, are theoretically possible: cis- or trans-S-(1, 2-dichlorovinyl)glutathione (cis- or trans-1,2-DCVG, respectively) or 2,2-DCVG. This study involved application of ab initio molecular orbital theory to computing potential energy profiles (PEPs) and predicting product outcome of the reaction of CH3S- with TCE as a model for GSH-TCE conjugation in biological systems. A goal of this study was to determine the extent to which a body of chemical knowledge pertaining to nucleophilic vinylic substitution (SNV) reactions, of which the GSH-TCE conjugation is a representative example, is relevant to this biological conjugation problem. PEPs were computed for all studied species at the HF/6-31+G level of theory; electron correlation effects were estimated at the MP2/6-31+G and MP4/6-31+G levels, and the influence of solvation was estimated using the PS-GVB solvation model. Multiple proposed reaction pathways were considered, including conjugation at the C1 or C2 site on TCE, by in-plane (sigma) or out-of-plane (pi) approach of the nucleophile. Some aspects of the MP2 and HF PEPs were found to differ significantly. However, on the basis of comparison of activation barriers, calculations at all levels of theory predict preference for C2 conjugation over C1 conjugation and formation of the trans-1,2-DCVM product over the cis-1,2-DCVM product. These predictions are consistent with GSH-TCE conjugation results from in vivo experiments. In contrast, relative product energies appear to be a poor indicator of the product outcome for this system. Hence, theoretical consideration of the reaction chemistry in the vicinity of the site of

nucleophilic addition appears to be necessary and sufficient to predict the outcome of the enzyme-mediated GSH-TCE conjugation.

Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW, Walker NJ. **Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes.** Cancer Epidemiol Biomarkers Prev 1999;8 (2):139-46.
Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) results in a broad spectrum of biological responses, including altered metabolism, disruption of normal hormone signaling pathways, reproductive and developmental effects, and cancer. Cytochrome P450 1B1 (CYP1B1) is a dioxin-inducible gene that is active in the formation of 4-hydroxyestradiol, a potentially genotoxic catechol estrogen. Therefore, the analysis of CYP1B1 in humans may be useful in establishing relationships between dioxin exposure and adverse health effects. In this study, we examined the expression of CYP1B1 in human peripheral blood lymphocytes of unexposed individuals using a quantitative reverse transcription-PCR method. Absolute CYP1B1 RNA levels varied more than 30-fold in uncultured mononuclear cells obtained from 10 individuals. In vitro treatment of mitogen-stimulated lymphocytes with TCDD for 1-5 days of culture resulted in a peak induction of CYP1B1 after 3 days. The induction of CYP1B1 RNA levels after 3 days of culture was dose-dependent, exhibited a maximum response above 10 nM TCDD, and varied greatly among different individuals. However, the half maximal dose required for this induction was similar between individuals and comparable to that observed in the MCF-7 and HepG2 human cell lines. These observations indicate that CYP1B1 exhibits variable constitutive expression and is inducible in vitro by TCDD in human lymphocytes and that the magnitude of induction varies within the population. These data define the suitability of CYP1B1 for use as a mechanistically based biomarker in ongoing molecular epidemiological studies of human populations exposed to dioxins and related chemicals that bind the aromatic hydrocarbon receptor.

Stensrud G, Passi S, Larsen T, Sandset PM, Smistad G, Monkkonen J, Karlsen J. **Toxicity of gamma irradiated liposomes. 1. In vitro interactions with blood components.** Int J Pharm 1999;178(1):33-46.
Gamma irradiation is a potential technique for sterilisation of liposome suspensions. Unfortunately, gamma irradiation may result in chemical degradation of the phospholipids and the toxicological aspects have to be considered. The effects of liposome composition and gamma irradiation on the interactions of the liposomes with the hemostatic mechanisms (hemolysis, aggregation and coagulation) were studied. Non-irradiated liposome suspensions showed no hemolysis of erythrocytes. After irradiation, up to 3.1% hemolysis was measured. Least hemolysis was observed with irradiated liposomes composed of unsaturated or charged phospholipids. The negatively charged DSPG-liposomes (both non-irradiated and irradiated) induced aggregation of platelets as observed by the spectrophotometric method. However, no aggregates were seen in the microscope or measured by the aggregometer. Negatively charged liposomes also affected the coagulation cascade where prolonged coagulation times were measured. Irradiation of the liposome suspensions resulted in even longer coagulation times. The prolonged coagulation times correlated to some extent with the measured binding and depletion of calcium from plasma by the negatively charged liposomes.

Suzuki T, Ezure T, Ishida M. **Synergistic effects of some pairs of antioxidants and related agents on mouse leukaemia L5178Y cell growth in-vitro.** J Pharm Pharmacol 1998;50(10):1173-7.
The effects of simultaneous administration of some dyadic combinations of antioxidants or vitamins and related agents on cellular proliferation of mouse leukaemia L5178Y cells in-vitro have been examined experimentally. The data were analysed on the basis of the concept of independence for evaluation of interactions between biologically active agents. An approach for evaluation of the synergism or antagonism of the action of two agents is proposed in which the types and extents of interactions are described by response-surface diagrams. The combinations phytol with trans-retinol, abscisic acid with trans-retinol, and menadione with sodium L-ascorbate were synergistic, whereas menadione with trans-retinol, and plumbagin with trans-retinol were antagonistic in the dose-range tested. These results reveal that the interactions between two agents depend not only on the combinations of agents but also on the dose ranges or the ratios of agents under the experimental domain studied.

Tateishi T, Watanabe M, Nakura H, Tanaka M, Kumai T, Aoki T, Kobayashi S. **A comparison of the inhibitory effects of four volatile anaesthetics on the metabolism of chlorzoxazone, a substrate for CYP2E1, in rabbits.** Acta Anaesthesiol Scand 1998;42(9):1028-32.
BACKGROUND: Halothane inhibits in vitro and in vivo activity of cytochrome P-450 (CYP) 2E1. There are several fluorinated volatile anaesthetics besides halothane, and most of them are defluorinated by CYP2E1. It is unclear whether other fluorinated anaesthetics inhibit the in vivo activity of CYP2E1. METHODS: We compared the inhibitory effects of therapeutic concentrations of four inhalational anaesthetics, halothane, enflurane, isoflurane, and sevoflurane, on chlorzoxazone metabolism in rabbits receiving artificial ventilation. RESULTS: All four inhalational anaesthetics decreased arterial blood pressure and increased plasma chlorzoxazone concentration. However, no significant differences in the plasma chlorzoxazone concentration were found between the four anaesthetics. The estimated chlorzoxazone clearance increased after beginning inhalation with all four agents, but no significant difference in clearance was noted between agents. CONCLUSIONS: At therapeutic concentrations, the in vivo inhibitory effect on chlorzoxazone metabolism was similar for all four inhalational anaesthetics examined, even though their chemical characteristics and extent of hepatic metabolism differ considerably.

Tod M, Rocchisani JM. **Comparison of ED, EID, and API criteria for the robust optimization of sampling times in pharmacokinetics**. J Pharmacokinet Biopharm 1997 Aug;25:515-37.
IPA COPYRIGHT: ASHP The respective performances of 3 expectation-based optimal design methods for the robust optimization of sampling times in pharmacokinetic studies in comparison to the D-optimal design method and a naive sampling schedule were studied using a large number of simulated data sets for a 1-compartment model with a first-order absorption rate (3 parameters) and a 2-compartment model with a zero-order infusion rate (4 parameters). The optimal design methods included those that maximize the expected value (over a given parameter distribution) of the determinant of the Fisher information matrix (det FIM) (ED optimal design) or the logarithm of the det FIM (API optimal design) or those that minimize the expectation of the inverse of the det FIM (EID optimal design). Compared to the D-optimal design method, the EID and API optimal design methods reduced the bias and imprecision of the estimation of parameters having large interindividual variability. In some cases, the API optimal design method resulted in a higher number of acceptable estimates.

Ulrich H, Ippolito JE, Pagan OR, Eterovic VA, Hann RM, Shi H, Lis JT, Eldefrawi ME, Hess GP. **In vitro selection of RNA molecules that displace cocaine from the membrane-bound nicotinic acetylcholine receptor.** Proc Natl Acad Sci U S A 1998;95(24):14051-6.
The nicotinic acetylcholine receptor (AChR) controls signal transmission between cells in the nervous system. Abused drugs such as cocaine inhibit this receptor. Transient kinetic investigations indicate that inhibitors decrease the channel-opening equilibrium constant [Hess, G. P. & Grewer, C. (1998) Methods Enzymol. 291, 443-473]. Can compounds be found that compete with inhibitors for their binding site but do not change the channel-opening equilibrium? The systematic evolution of RNA ligands by exponential enrichment methodology and the AChR in Torpedo californica electroplax membranes were used to find RNAs that can displace inhibitors from the receptor. The selection of RNA ligands was carried out in two consecutive steps: (i) a gel-shift selection of high-affinity ligands bound to the AChR in the electroplax membrane, and (ii) subsequent use of nitrocellulose filters to which both the membrane-bound receptor and RNAs bind strongly, but from which the desired RNA can be displaced from the receptor by a high-affinity AChR inhibitor, phencyclidine. After nine selection rounds, two classes of RNA molecules that bind to the AChR with nanomolar affinities were isolated and sequenced. Both classes of RNA molecules are displaced by phencyclidine and cocaine from their binding site on the AChR. Class I molecules are potent inhibitors of AChR activity in BC3H1 muscle cells, as determined by using the whole-cell current-recording technique. Class II molecules, although competing with AChR inhibitors, do not affect receptor activity in this assay; such compounds or derivatives may be useful for alleviating the toxicity experienced by millions of addicts.

Wu Y, Patterson C. **The human KDR/flk-1 gene contains a functional initiator element that is bound and transactivated by TFII-I.** J Biol Chem 1999;274(5):3207-14.
KDR/flk-1, the receptor for vascular endothelial growth factor, is required for normal vascular development. KDR/flk-1 is a TATA-less gene, containing four upstream Sp1 sites and a single transcription start site, although analysis of the start site sequence discloses only weak similarities with the consensus initiator element (Inr) sequence. In vitro transcription assays, however, demonstrate that the region from -10 to +10 relative to the start site contains Inr activity that is orientation- and position-dependent, and mutagenesis of the KDR/flk-1 Inr reduces promoter activity to 28% of the wild-type promoter in transient transfection assays. Gel shift assays confirm that nuclear proteins specifically bind the Inr, and competition experiments demonstrate that TFII-I, a multifunctional Inr-binding nuclear protein, is a component of these DNA-protein complexes. TFII-I transactivates the wild-type KDR/flk-1 promoter, but not a promoter containing a mutated Inr, in transient transfection assays. Immunodepletion of TFII-I from nuclear extracts prior to in vitro transcription assays abolishes transcription from the KDR/flk-1 Inr, an effect that can be rescued by adding back purified TFII-I, reflecting the importance of TFII-I in KDR/flk-1 Inr activity. These experiments demonstrate that the KDR/flk-1 gene contains a functional Inr that is bound by TFII-I and that both the functional Inr and TFII-I activity are essential for transcription.

Yu LX, Amidon GL. **Saturable small intestinal drug absorption in humans: modeling and interpretation of cefatrizine data**. Eur J Pharm Biopharm 1998;45(2):199-203.
IPA COPYRIGHT: ASHP An extended compartmental absorption and transit (CAT)-based

pharmacokinetic model for estimating saturable small intestinal absorption in humans that simultaneously considers passive absorption, saturable absorption, degradation, and transit kinetics in the small intestine is described; the model was validated using cefatrizine, based on oral doses of 250, 500, and 1000 mg of this agent. The extended CAT-based pharmacokinetic model was able to interpret the dose-dependent pharmacokinetics of cefatrizine. There was comparable passive and saturable absorption of cefatrizine, particularly at high doses.

Yu X, Johanson G, Ichihara G, Shibata E, Kamijima M, Ono Y, Takeuchi Y. **Physiologically based pharmacokinetic modeling of metabolic interactions between n-hexane and toluene in humans.** J Occup Health 1998;40(4):293-301.
BIOSIS COPYRIGHT: BIOL ABS. Some animal experiments have shown that mutual metabolic inhibition takes place between n-hexane and toluene, but we have found only one report dealing with their metabolic interaction at occupationally relevant exposure levels (Baelum et al. 1998). In order to evaluate the effect of dose-dependent metabolic interaction between toluene and n-hexane, especially in occupationally relevant exposure conditions such as relevant exposure levels, physical activities and exposure patterns, a physiologically based pharmacokinetic (PBPK) model for co-exposure to n-hexane and toluene was developed. The PBPK model for the binary co-exposure was established by initially validating or refining the existing PBPK models for n-hexane and toluene and then linking the individual solvent models via the hepatic metabolism terms. In reporting previous findings, noncompetitive inhibition was assumed and the inhibition constant of toluene on n-hexane biotransformation and that of n-hexane on toluene biotransformation used in simulation were 7.5, 30 mu, respectively, in previous data. According to the model, 8 h of constant exposure to 50 ppm n-hexane and 25, 50, 100 and 500 ppm toluene will cause about 7%, 18%, 62% and 96% decreases in the urinary excretion of 2,5-hexanedione (2,5-HD) and 4%, 10%, 25% and 30% increases in the n-hexane concentration in blood at the end of the fifth day of exposure simulated in a standard man at a 25 W work load. Simulations of co-exposure to 50 ppm n-hexane and 50 ppm toluene in a standard man who inhaled 50 ppm n-hexane with 0 or 50 ppm toluene for 8 h at different work loads suggest that toluene causes a slight decrease in urinary 2,5-HD in the resting condition, a 17% decrease at 25 W, and a 41% decrease at 50 W work load. The simulations of co-exposure in different exposure patterns with the same time-weighted concentration (TWA) of 50 ppm, i.e. 50 ppm for 8 h, 100 ppm of 4 times for 1 h and 200 ppm of twice for 1 h, showed reductions in urinary 2,5-HD of 17%, 40% and 67%, respectively. These simulations suggest that co-exposure to n-hexane and toluene around 50 ppm (TWA) could affect urinary n-hexane metabolites to various degrees depending on the fluctuations in exposure concentrations and variety of work activities in the workplace.

Zeevalk GD, Bernard LP, Sinha C, Ehrhart J, Nicklas WJ. **Excitotoxicity and oxidative stress during inhibition of energy metabolism.** Dev Neurosci 1998;20(4-5):444-53.
Glutamate receptor involvement and oxidative stress have both been implicated in damage to neurons due to impairment of energy metabolism. Using two different neuronal in vitro model systems, an ex vivo chick retinal preparation and dopamine neurons in mesencephalic culture, the involvement and interaction of these events as early occurring contributors to irreversible neuronal damage have been examined. Consistent with previous reports, the early acute changes in the retinal preparation, as well as irreversible loss of dopamine neurons due to inhibition of metabolism, can be prevented by blocking

NMDA receptors during the time of energy inhibition. Oxidative stress was suggested to be a downstream consequence and contributor to neuronal cell loss due to either glutamate receptor overstimulation or metabolic inhibition since trapping of free radicals with the cyclic nitrone spin-trapping agent MDL 102,832 (1 mM) attenuated acute excitotoxicity in the retinal preparation or loss of mesencephalic dopamine neurons due to either metabolic inhibition by the succinate dehydrogenase inhibitor, malonate, or exposure to excitotoxins. In mesencephalic culture, malonate caused an enhanced efflux of both oxidized and reduced glutathione into the medium, a significant reduction in total reduced glutathione and a significant increase in total oxidized glutathione at time points that preceded those necessary to cause toxicity. These findings provide direct evidence for early oxidative events occurring following malonate exposure and suggest that the glutathione system is important for protecting neurons during inhibition of energy metabolism. Consistent with this, lowering of glutathione by buthionine sulfoxamine (BSO) pretreatment greatly potentiated malonate toxicity in the mesencephalic dopamine population. In contrast, BSO pretreatment did not potentiate glutamate toxicity. This latter finding indicates dissimilarities in the type of oxidative stress that is generated by the two insults and suggests that the oxidative challenge during energy inhibition is not solely a downstream consequence of glutamate receptor overstimulation.

PULMONARY TOXICITY

Bhalla DK. **Ozone-induced lung inflammation and mucosal barrier disruption: toxicology, mechanisms, and implications.** J Toxicol Environ Health B Crit Rev 1999;2(1):31-86.
The airway epithelial lining serves as an efficient barrier against penetration of exogenous particles and macromolecules. Disruption of this barrier following O3 exposure represents a state of compromised epithelial defenses leading to increased transmucosal permeability. Although the barrier disruption following an acute exposure is transient in nature, the brief period of disruption caused by O3, an oxidant air pollutant, provides an opportunity for facilitated entry of a potentially toxic particulate copollutant(s) across the airway epithelia. The subsequent deposition and retention of the copollutant(s) in the subepithelial compartment for prolonged periods adds the risk of injury due to chronic exposure following an acute episode. Toxicological studies from several laboratories have demonstrated alterations in epithelial permeability, suggestive of barrier disruption, in animals and humans exposed to O3. Inflammatory cells represent another important component of pulmonary defenses, but upon activation these cells can both induce and sustain injury. The recruitment of these cells into the lung following O3 exposure presents a risk of tissue damage through the release of toxic mediators by activated inflammatory cells. Several studies have reported concomitant changes in permeability and recruitment of the inflammatory cells in the lung following O3 exposure. In these studies, an inflammatory response, as detected by an increase in the number of polymorphonuclear leukocytes in the bronchoalveolar lavage (BAL) or in lung parenchyma, was accompanied by either an increased tracer transport across the airway mucosa or an elevation in the levels of total protein and/or albumin in the BAL. The magnitude of response and the time at which the permeability changes and inflammatory response peaked varied with O3 concentration, exposure duration, and the mode of analysis. The responsiveness to O3 also appeared to vary with the animal species, and increased under certain conditions such as physical activity and pregnancy. Some of the effects seen after an acute exposure to O3 were modified upon repeated exposures. The responses following repeated exposures included

attenuation, persistence, or elevation of permeability and inflammation. Mechanistic studies implicate chemotactic factors, cellular mediators, and cell-surface-associated molecules in the induction of inflammation and lung injury. In discussing these studies, this review serves to introduce the mucosal barrier functions in the lung, evaluates inflammatory and permeability consequences of O3, addresses mechanisms of inflammatory reactions, and offers alternate viewpoints.

Dejongh J, Verhaar HJ, Hermens JL. **Role of kinetics in acute lethality of nonreactive volatile organic compounds (VOCs).** Toxicol Sci 1998;45(1):26-32.
The role of kinetics in the acute inhalation toxicity of nonreactive, volatile organic compounds (VOCs), including lipophilic and hydrophilic compounds, was analyzed with a physiologically based pharmacokinetic (PB-PK) model for the rat. For 15 VOCs, a total of 23 LC50 values were retrieved from the literature. It was observed that the external exposure parameter (LC50.exposure length; in ppm. h), varied approximately 60-fold. Concentrations of compounds in the lipoid brain fraction were simulated using a kinetic model. This lead to a more than 10-fold reduction in the toxic range of the 15 VOCs. The average value for this simulated dose surrogate was 70 +/- 31 mM for all VOCs. These observations support the presumption that nonspecific, acute narcotic lethality is directly related to the extent of VOC distribution into lipoid brain constituents. The present results can be used for estimation of the acute lethality of nonreactive VOCs on the basis of kinetic simulations. In addition, the presently calculated dose surrogate for VOC lethality in rats is found to be very similar to the reported internal lethal concentrations of so-called "baseline toxicity compounds" in fish. This indicates a common mechanism of acute VOC toxicity among mammalian and aquatic species.

Stone V, Shaw J, Brown DM, Macnee W, Faux SP, Donaldson K. **The role of oxidative stress in the prolonged inhibitory effect of ultrafine carbon black on epithelial cell function.** Toxicol In Vitro 1998;12(6):649-59.
BIOSIS COPYRIGHT: BIOL ABS. Respired ultrafine particles induce a greater inflammation in rat lungs than fine particles; we have hypothesized that this is due to their comparatively huge number and surface area for the production of free radicals. We tested this hypothesis by studying the effects of fine and ultrafine (uf) carbon black (CB) particles in comparison with quartz on A549 human type II alveolar epithelial cells, particularly with respect to the oxidative properties of these particles. Treatment with fine CB (diameter 260 mm), and quartz (up to 0.78 mug/mm2) for 24 hours significantly ($P < 0.05$) decreased the A549 cells metabolic competence, as measured by the ability to reduce MTT to a formazan product. The inhibitory effects of uf CB only became significantly different ($P < 0.05$) relative to the control at 48 hours, by which time the effects of fine CB and quartz were no longer significant. The inhibition of MTT reduction by uf CB was prevented by the hydroxyl radical scavenger mannitol (2 mM). In addition, measurement of reactive oxygen species production using supercoiled plasmid DNA showed that uf CB exhibited significantly more free radical activity than fine CB ($P < 0.05$). In the absence of serum, uf CB depleted reduced glutathione at 6 hours ($P < 0.008$). In contrast, CB did not significantly alter reduced or oxidized glutathione. Hence, compared with fine CB, uf CB exhibited greater free radical activity, greater inhibition of the reduction of MTT at 48 hours (prevented by mannitol) and a depletion of reduced glutathione. These results suggest that uf CB induces a greater oxidative stress than fine CB, and that this may play a role in the toxicological effects of this ultrafine particle.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Ali HM, Mostafa AA. **Quantitative structure-activity relationship of a series of N-aryl O-aryl phosphoramidate insecticides.** Environ Toxicol Chem 1999;18(2):167-71.
BIOSIS COPYRIGHT: BIOL ABS. A series of O-ethyl O-aryl N-aryl phosphoramidates was prepared and examined for housefly contact toxicity and acetylcholinesterase (AChE) inhibition. Results showed a moderate toxicity and enzyme inhibition effect. These biological properties correlated to Hammett sigma constants in the direction of increasing activity with increasing electron donation of the substituents on either the phenolic or anilinic ring of the phosphoramidates. These correlations suggest the formation of a positive charge on the phosphorus atom that is stabilized by electron-donating groups. This result invokes the mechanism of the electrophilic attack of phosphorus on a nucleophilic center of AChE. The moderate toxicity of phosphoramidates is attributed to the reduction of the positive charge on the phosphorus atom by the effect of the neighboring nitrogen atom during the course of the enzyme-inhibitor interaction and hence decreasing the inhibition effect. Mass spectra of these compounds are also discussed in some detail.

Barratt MD. **Integrating computer prediction systems with in vitro methods towards a better understanding of toxicology.** Toxicol Lett 1998;102-103:617-21.
Structure Activity Relationships (SARs) or Quantitative Structure Activity Relationships (QSARs) form the basis of most computer prediction systems in toxicology. The underlying premise of SARs and QSARs is that the properties of a chemical are implicit in its molecular structure. For an SAR or QSAR to be valid and reliable, the dependent property for all of the chemicals covered by the relationship has to be elicited by a mechanism which is both common to the set of chemicals as well as relevant to that dependent property. Similar principles must also be applied to the development of in vitro alternatives to animal tests if those methods are to be reliable. A number of ways in which computer prediction systems and in vitro toxicology can complement each other in the development of alternatives to live animal experiments are described.

Bearden AP, Schultz TW. **Comparison of Tetrahymena and Pimephales toxicity based on mechanism of action.** SAR QSAR Environ Res 1998;9(3-4):127-53.
The toxicity data of 256 chemicals tested in both the 96-h Pimephales promelas mortality assay and the 2-d Tetrahymena pyriformis growth inhibition assay were evaluated using quantitative structure-activity relationships (QSARs). Each chemical was a priori assigned a mode of action of either narcoses or soft electrophilicity. Narcoses were separated into nonpolar narcosis, polar narcosis, monoester narcosis, diester narcosis, amine narcosis, and weak acid respiratory uncoupling based on the presence or absence of specific toxicophores. Toxicity of each narcotic mechanism was initially regressed against the 1-octanol-water partition coefficient (log K(ow)). The slopes of these log K(ow) based QSARs were observed to ascertain whether a relationship exists between the value of the slope and the reactivity of the mechanism of action. With both the fish and ciliate data nonpolar narcosis was the least reactive mechanism. It was followed by the other reversible narcoses. The soft electrophile mode was separated into the specific molecular mechanisms of: SN2 reactors, Schiff-base formers, Michael-type addition, or proelectrophilicity (precursors to Michael-type addition chemicals). These mechanisms were represented

structurally by the nitrobenzenes, aldehydes, polarized alpha-beta unsaturates (e.g., acrylates and methacrylates), and acetylenic alcohols, respectively. Electrophilic toxicity was not correlated with hydrophobicity. QSARs based on molecular orbital (MO) quantum chemical descriptors were used to improve the predictability of the electrophilic mechanisms. Relevant descriptors include average superdelocalizability (Svna) for the nucleophilic addition of the nitrobenzene; atom x and y acceptor superdelocalizability (Ax); and bond order (Bx y) for the Michael-type addition of the acrylates; and log K(ow) and atom x net charge (Qx) for the Schiff-base forming aldehydes. The pertinent descriptors for proelectrophiles were log K(ow) and Svna. Principal differences between the QSARs for the two biological endpoints were observed for the ester narcoses, proelectrophiles, and Schiff-base forming aldehydes.

Bohnenstengel FI, Steube KG, Meyer C, Nugroho BW, Hung PD, Kiet LC, Proksch P. **Structure activity relationships of antiproliferative rocaglamide derivatives from Aglaia species (Meliaceae).** Z Naturforsch [C] 1999;54(1-2):55-60.
Eleven rocaglamide derivatives (cyclopentatetrahydrobenzofurans) and one structurally related aglain congener all isolated from different Aglaia species (Meliaceae) were tested for growth inhibiting properties using the human cancer cell lines MONO-MAC-6 and MEL-JUSO. Proliferation of both cell lines was efficiently inhibited in a dose and compound dependent manner. Applying MTT-Assay, the IC50 of the most active compound didesmethyl-rocaglamide (1) was observed at 0.002 and 0.006 micrograms/ml (0.004 and 0.013 microM) depending on the cell line investigated. Bulky aminoacyl substituents at C-2, acetylation of the OH substituent at C-1 or insertion of a OH or OMe substituent at C-3 of the rocaglamide skeleton all diminished the activity of the compounds investigated. The aglain derivative 12 was inactive up to a concentration of 3 micrograms/ml (4.6 microM). This loss of activity is assumed to be mainly due to the presence of a pyran ring in the aglains vs. a furan ring as found in rocaglamide derivatives. Rocaglamide derivatives may act primarily by inhibition of cell proliferation as evidenced by the absence of a significant cytotoxic effect in long-term cultures of MONO-MAC-6 cells treated with high doses of didesmethylrocaglamide. Our data suggest that rocaglamide derivatives could exert a potential role in the treatment of malignant diseases and are worth to be investigated in further studies of experimental medicine and pharmacology.

Borek V, Elberson LR, McCaffrey JP, Morra MJ. **Toxicity of isothiocyanates produced by glucosinolates in brassicaceae species to black vine weevil eggs.** J Agric Food Chem 1998;46 (12):5318-23.
BIOSIS COPYRIGHT: BIOL ABS. Control of the black vine weevil, Otiorhynchus sulcatus (F.), with allelochemicals produced from glucosinolates may be possible; however, plant-derived isothiocyanates are not readily available for bioassays. Our objective was to predict the toxicity of plant-derived isothiocyanates using a model developed with commercially available compounds. Contact toxicities of 12 organic isothiocyanates were determined by dipping black vine weevil eggs into isothiocyanate solutions. Quantitative relationships between the molecular structure of the isothiocyanates and their toxicities were estimated by regressing lethal concentrations against the compound's respective physiochemical parameters. Isothiocyanate polarity (log octanol/water partition coefficient) had the most significant effect on observed toxicities, whereas electronic and steric characteristics were unimportant. Using this linear structure-activity relationship, we predict that the highest contact toxicities to black

vine weevil eggs will result from glucosinolates producing isothiocyanates with higher numbers of carbon atoms or those bearing sulfinyl thio or aromatic moieties.

Chilmonczyk Z, Ksycinska H, Polec I. **Application of chiral chromatographic parameters in quantitative structure-activity relationship analysis of homologous malathion derivatives.** J Chromatogr B Biomed Sci Appl 1998;720(1-2):65-9.
The conditions of the chiral resolution of the racemic malathion O,O-di-n-alkyl derivatives on cellulose tris(3,5-dimethylphenylcarbamate) are described. Quantitative relationships between chromatographic parameters obtained on chiral and achiral stationary phases and acute toxicity of the compounds towards house fly are derived and discussed.

Cho WJ, Kim EK, Park MJ, Choi SU, Lee CO, Cheon SH, Choi BG, Chung BH. **Synthesis and comparative molecular field analysis (CoMFA) of antitumor 3-arylisoquinoline derivatives.** Bioorg Med Chem 1998;6(12):2449-58.
In this study a series of 3-arylisoquinoline derivatives were synthesized and cytotoxicity against human melanoma tumor cell evaluated, and a three dimensional quantitative structure-activity relationship was investigated using the comparative molecular field analysis (CoMFA). The results suggested that the electrostatic, steric and hydrophobic factors of 3-arylisoquinolines were strongly correlated with the antitumor activity. Considerable predictive ability (cross-validated r2 as high as 0.721) was obtained through CoMFA.

Demeter DA, Weintraub HJ, Knittel JJ. **The local minima method (LMM) of pharmacophore determination: a protocol for predicting the bioactive conformation of small, conformationally flexible molecules.** J Chem Inf Comput Sci 1998;38(6):1125-36.
Software has been developed for potential energy surface analysis and the local minima method of pharmacophore determination. LMM is rigorous and systematic and employs multiple conformations which are the local minima from the potential energy surface of each compound in the data set. It produces a series of possible pharmacophores from a postulated set of pharmacophore elements. The best pharmacophore is then determined by performing a comparative molecular field analysis (CoMFA) on each one. The pharmacophore which produces the most self-consistent model is deemed the best. Local minima on the gas-phase potential energy surface are shown to be a reasonably close approximation to protein bound conformations, and these conformations can be found through systematic conformational searches followed by minimization of the local minima. LMM was used to develop a 3D-QSAR model for dopamine beta-hydroxylase (DBH) inhibitors which was highly predictive (predictive R2 = 0.71 and standard error of predictions = 0.41). The model predicted that the phenyl and thienyl series of inhibitors were acting as bioisosteres. Examination of compounds overlayed in the model indicated a possible hydrogen bond acceptor in the DBH active site. Three tyrosine residues previously labeled by mechanism based inhibitors may be acting as the acceptor and therefore represent excellent candidates for site-directed mutagenesis studies.

Doytchinova I, Natcheva R. **QSAR study on a series of 1,4-disubstituted piperazines with analgesic activity**. Acta Pharm 1997;47(3):189-95.
IPA COPYRIGHT: ASHP The quantitative structure-activity relationships of a series of 1,4-

disubstituted piperazines with analgesic activity are reported. It was found that the weak steric hindrance and the low electron-withdrawing effect of the substituents at N1 and N4 increase both the analgesic activity and the toxicity of the newly synthesized 1,4-disubstituted piperazines. Also, symmetry in these structures increases the activity as well.

Durst GL. **Comparative molecular field analysis (CoMFA) of herbicidal protoporphyrinogen oxidase inhibitors using standard steric and electrostatic fields and an alternative LUMO field.** Quant Struct Activity Relat 1998;17(5):419-26.
BIOSIS COPYRIGHT: BIOL ABS. A set of diphenyl ether herbicides (1) was examined with Comparative Molecular Field Analysis (CoMFA) using standard steric and electrostatic fields and alternative frontier orbitals as 3-D fields to explain observed Protoporphyrinogen oxidase (PPO) enzyme inhibition. Significant CoMFA models were obtained utilizing standard CoMFA and the LUMO field both together and separately. These findings support previous QSAR work that identified several electronic properties as important for PPO inhibition. The resulting CoMFA models identify specific 3-D steric and electronic interactions affecting PPO enzyme binding most significantly.

Estrada E. **Structure-mutagenicity relationships in 2-furylethylene derivatives. A molecular orbital study of the role of nitro groups.** Mutat Res 1998;420(1-3):67-75.
An analysis of the electronic molecular structure of 2-furylethylene derivatives is carried out using a molecular orbital method. The differences in the electronic features of the nitro groups at two different positions of the furylethylene framework are well-accounted for in the present study. It is shown that nitro groups at position 5 of the furan ring of these compounds are more sensible to reduction in biological media that those at position beta of the exocyclic double bond. This greater sensitivity to reduction of 5-nitro compounds is given by the atomic and group charges on nitro group as well as for the low values of the energy of the lowest unoccupied molecular orbital. Discriminant functions able to classify 2-furylethylenes as mutagenic or not mutagenic are also obtained. These functions permitted the classification of 2-furylethylenes having or not nitro group in their structures. Finally, some quantitative models that describe the mutagenic potency of active compounds in terms of electronic molecular parameters were obtained. In all cases the models developed are in complete agreement with the experimental findings reported for this kind of compounds. Copyright 1998 Elsevier Science B.V.

Ford GP, Thompson JW. **Regiochemistry of nucleophilic attack by the guanine 2-amino group at the ring positions of nitrenium ions derived from carcinogenic polycyclic arylamines and nitroarenes: molecular orbital calculations and simple models.** Chem Res Toxicol 1999;12(1):53-9.
Semiempirical AM1 molecular orbital calculations are used to compute the energetics of addition of the guanine 2-amino group to alternative ring positions of aryl nitrenium ions with the general structure ArNH+, where Ar is the phenyl and various positional isomers of the naphthyl, pyrenyl, and benzo[a] pyrenyl groups. The syn or anti orientation of the NH+ group, and factors akin to classical localization energies, are identified as key components of the differential energetics of addition to alternative ring sites. The regiochemistry predicted by the AM1 method can be qualitatively reproduced using simple HMO calculations that require trivial computational effort and, almost as well, using PMO theory that does not require the use of a computer at all. In the latter approach, the most reactive ring positions are predicted to be those where the nonbonding orbital coefficients, a0r, in the analogous odd alternant

hydrocarbons are largest. These results are discussed in relation to the available experimental data for the formation of deoxyguanosin-2-yl adducts when DNA is exposed to presumed nitrenium ion precursors.

Gao H, Denny WA, Garg R, Hansch C. **Quantitative structure-activity relationships (QSAR) for 9-anilinoacridines: a comparative analysis.** Chem Biol Interact 1998;116(3):157-80.
A new analysis of the quantitative structure-activity relationship (QSAR) of the antitumor activity of anilinoacridines against L1210 leukemia in mice and mouse toxicity is reported. QSAR have also been derived for the inhibitory activity of the anilinoacridines with tumor cells and their binding to DNA. These results are compared with reactivity with simple nucleophiles. The comparative analysis shows the importance of electron releasing substituents (in general negative coefficients with the Hammett parameter sigma+) throughout the various systems and the complete lack of hydrophobic interactions from DNA to cells to mice. The presence of steric terms suggests that a protein receptor is involved. The study shows that QSAR has an important role to play in improving the efficiency in the design of bioactive compounds and that care must be taken in the design of a set of congeners so that the necessary parameters are available to do the QSAR analysis. Our study illustrates the value of comparative QSAR in generalizing our understanding of chemical-biological interactions.

Gramatica P, Consonni V, Todeschini R. **QSAR study on the tropospheric degradation of organic compounds.** Chemosphere 1999;38(6):1371-8.
BIOSIS COPYRIGHT: BIOL ABS. In the environmental risk assessment of organic chemicals, persistence is of particular importance as it may lead to adverse effects. The reaction of chemicals with OH and NO3 radicals and ozone are the main abiotic degradation processes in the troposphere, so an upper limit of the atmospheric persistence of chemicals is assessed by determining their reaction rate constants with OH. and NO3. and O3. Statistical models predicting the oxidation rate constants with OH. and NO3. for many heterogeneous compounds have been developed by the QSAR/QSPR (Quantitative Structure-Activity/Property Relationships) approach; the structural representation of the compounds was realized using different kinds of molecular descriptors (structural, topological, empirical and WHIM descriptors). In addition, Kohonen neural networks (K-ANN) and the GA-VSS (Genetic Algorithm Variable Subset Selection) strategy were respectively used to select the most representative training set and the best descriptor subset. The predictive capability of the models on kOH and kNO3 has been checked and appears to be satisfactory. Finally, the oxidation rate constants for some chemicals of concern were analysed in the Principal Component space in order to rank these chemicals according to their tropospheric degradability.

Guesten H. **Predicting the abiotic degradability of organic pollutants in the troposphere.** Chemosphere 1999;38(6):1361-70.
BIOSIS COPYRIGHT: BIOL ABS. Based on a global average of the OH and NO3 free radial concentration in the troposphere, the lifetime of organic chemicals can be calculated from the rate constant of their reaction with the free radicals. Various models for estimating the reactivity of organic compounds with the tropospheric free radicals allow a rapid estimation of their degradability. An overview on the existing models - empirical, quantitative structure-activity relationships (QSAR) with measured physico-chemical descriptors, QSAR with semiempirical quantum-chemical descriptors as

well as ab initio molecular orbital calculations - is described and their limitations and range of applicability to estimate the tropospheric lifetime of an organic compound is discussed.

Hall AH. **Computer modeling and computational toxicology in new chemical and pharmaceutical product development.** Toxicol Lett 1998;102-103:623-6.
A theoretical basis for use of computer modeling and bioinformatics resources including the internet in decisions about whether to attempt synthesis and toxicology testing of new chemical or pharmaceutical products is described. Steps in the process include: (1) identification of a potentially efficacious chemical or pharmaceutical product; (2) structure-activity relationship (SAR) modeling; (3) synthesis methods and cost screening; (4) market screening for potential revenues; (5) regulatory impacts screening; (6) toxicology modeling and screening; (7) decision making about whether to attempt synthesis and testing. Some such computer modeling and screening processes are already in use. Others may reasonably be expected to be adopted in the near future. More development of structure-activity and structure-toxicity databases and therapeutic and toxicity molecular endpoints computerized libraries remains to be done. The internet is a rapidly developing source of information, but there are major problems with time-effectiveness, quality control, 'junk information' (misinformation), and deliberate 'disinformation'.

Hau KM, Connell DW, Richardson BJ. **Quantitative structure-activity relationships for nasal pungency thresholds of volatile organic compounds.** Toxicol Sci 1999;47(1):93-8.
A model was developed for describing the triggering of nasal pungency in humans, based on the partition of volatile organic compounds (VOCs) between the air phase and the biophase. Two partition parameters are used in the model: the water-air partition coefficient and the octanol-water partition coefficient. The model was validated using data from the literature, principally on alcohols, acetates and ketones. The model suggests that all test compounds, regardless of their chemical functional groups, bind to a common receptor site within the hydrophobic interior of the bilayer membrane of the trigeminal nerve endings. There is probably only a slight, non-specific interaction between the VOC molecule and the receptor molecule, whereas this type of non-specific interaction for the detection of odor is much stronger. In practical terms, the suggestion that all VOCs share a common irritation receptor site implies that nasal-pungency thresholds of individual VOCs may be additive. Quantitative structure-activity relationships (QSARs) for nasal-pungency thresholds were also developed from the model, which can be used to predict nasal-pungency thresholds of common VOCs. Although the present model does not offer additional precision over that of M.H. Abraham et al., 1996, Fundam. Appl. Toxicol. 31, 71-76, it requires fewer descriptors and offers a physiological basis to the QSAR. Another advantage of the present model is that it also provides a basis for comparison between the olfactory process and nasal pungency.

Hornak V, Balaz S, Schaper KJ, Seydel JK. **Multiple binding modes in 3D-QSAR: microbial degradation of polychlorinated biphenyls.** Quant Struct Activity Relat 1998;17(5):427-36.
BIOSIS COPYRIGHT: BIOL ABS. The published microbial degradation rates of nineteen polychlorinated biphenyls (PCB) were correlated with their structure and properties. A Free-Wilson-like calculation of the de novo binding energy contributions of the chlorine substituents was applied, considering simultaneous realization of up to four binding modes for each congener. Influence of the

PCB concentration in the vicinity of the degrading enzyme on the degradation rate was taken into account assuming pseudo-equilibrium PCB distribution in the microbial biomass. The resulting map of the binding site has two attractive regions and two repulsive regions positioned in the way that the PCB congeners bind to them in a non-planar conformation. The results are consistent with two commonly accepted perceptions of PCB degradation: (1) for majority of the studied congeners, the attack starts in non-chlorinated 2,3-position or equivalent position; (2) the less chlorinated phenyl ring is usually degraded first.

Kanazawa Y, Yoshida T, Kojima K. **Structure-activity relationships in allergic contact dermatitis induced by methacrylates. Studies of the influence of side-chain length of methacrylates.** Contact Dermatitis 1999;40(1):19-23.

Knight JL, Weaver DF. **A computational quantitative structure-activity relationship study of carbamate anticonvulsants using quantum pharmacological methods.** Seizure 1998;7(5):347-54.
A pattern recognition quantitative structure-activity relationship (QSAR) study has been performed to determine the molecular features of carbamate anticonvulsants which influence biological activity. Although carbamates, such as felbamate, have been used to treat epilepsy, their mechanisms of efficacy and toxicity are not completely understood. Quantum and classical mechanics calculations have been exploited to describe 46 carbamate drugs. Employing a principal component analysis and multiple linear regression calculations, five crucial structural descriptors were identified which directly relate to the bioactivity of the carbamate family. With the resulting mathematical model, the biological activity of carbamate analogues can be predicted with 85-90% accuracy.

Lepoittevin JP. **Development of structure-activity relationships (SARs) in allergic contact dermatitis.** Cell Biol Toxicol 1999;15(1):47-55.
One of the major objectives at the end of this century is the development of "alternative" tests for the evaluation of the pharmacological and/or toxicological activity of newly developed molecules. Contact allergy is no exception to the rule and many research programs have been started to develop in vitro techniques for the detection of allergizing compounds. In parallel with these biological studies, another approach is becoming important and will no doubt become more so, namely, the study of structure activity relationships (SARs). This consists of using molecular or physicochemical properties to predict and, in certain cases, quantify the allergizing potential of a new molecule without using any biological test. Three main approaches are currently under study: the creation of allergy databases, the design of "expert" computerized systems, and the development of quantitative SARs. These three often complementary approaches are still at the development stage, but we can begin to see their potential and limitations. The aim of this article is not to give an exhaustive description of all the systems developed worldwide, but to illustrate each approach by giving some important examples.

Lewis DF, Ioannides C, Parke DV. **A combined COMPACT and HazardExpert study of 40 chemicals for which information on mutagenicity and carcinogenicity is known, including the results of human epidemiological studies.** Hum Exp Toxicol 1998;17(10):577-86.
The COMPACT approach for defining structural criteria for substrates and inducers of cytochrome P450 (CYP) enzymes which mediate the formation of reactive intermediates is discussed in the context of

prediction of potential carcinogenicity. This is broadened to encompass structural studies on mammalian P450s, including those relevant to genetic polymorphism in man. The use of the COMPACT system, in parallel with the structure alert program HazardExpert (now incorporated into the Pallas system), for evaluating human carcinogenicity data is reported, as an example of the possible employment of a battery of short-term test procedures for safety evaluation. In particular, the importance of using the log P value (as a measure of compound lipophilicity) to assess the likelihood of a potentially toxic compound reaching the site of activation, is emphasized by the finding that most procarcinogens requiring metabolic activation by P450s are lipophilic in nature.

Matthews EJ, Contrera JF. **A new highly specific method for predicting the carcinogenic potential of pharmaceuticals in rodents using enhanced MCASE QSAR-ES software.** Regul Toxicol Pharmacol 1998;28(3):242-64.
This report describes in detail a new quantitative structure-activity relational expert system (QSAR-ES) method for predicting the carcinogenic potential of pharmaceuticals and other organic chemicals in rodents, and a beta-test evaluation of its performance. The method employs an optimized, computer-automated structure evaluation (MCASE) software program and new database modules which were developed under a Cooperative Research and Development Agreement (CRADA) between FDA and Multicase, Inc. The beta-test utilized 126 compounds with carcinogenicity studies not included in control database modules and three sets of modules, including: A07-9 (Multicase, Inc.), AF1-4 (FDA-OTR/Multicase, Inc.), and AF5-8 (FDA-OTR/proprietary). The investigation demonstrated that the standard MCASE(A07-9) system which had a small data-set (n = 319), detected few structure alerts (SA) for carcinogenicity (n = 17), and had poor coverage for beta-test compounds (51%). Conversely, the new, optimized FDA-OTR/MCASE(AF5-8) system had a large data-set (n = 934), detected many SA (n = 58) and had good coverage (94%). In addition, the study showed the standard MCASE(A07-9) software had poor predictive value for carcinogens and specificity for noncarcinogens (50 and 42%), detected many false positives (58%), and exhibited poor concordance (46%). Conversely, the new, FDA-OTR/MCASE(AF5-8) system demonstrated excellent predictive value for carcinogens and specificity for non-carcinogens (97%, 98%), detected only one false positive (2%), and exhibited good concordance (75%). The dramatic improvements in the performance of the MCASE were due to numerous modifications, including: (a) enhancement of the size of the control database modules, (b) optimization of MCASE SAR assay evaluation criteria, (c) incorporation of a carcinogenic potency scale for control compound activity and MCASE biophores, (d) construction of individual rodent gender- and species-specific modules, and (e) defining assay acceptance criteria for query and control database compounds.

Moysich KB, Mendola P, Schisterman EF, Freudenheim JL, Ambrosone CB, Vena JE, Shields PG, Kostyniak P, Greizerstein H, Graham S, et al. **An evaluation of proposed frameworks for grouping polychlorinated biphenyl (PCB) congener data into meaningful analytic units.** Am J Ind Med 1999;35(3):223-31.
BACKGROUND: Polychlorinated biphenyls (PCBs) have been associated with a variety of health outcomes. Enhanced laboratory techniques can provide a relatively large number of individual PCB congeners for investigation. However, to date there are no established frameworks for grouping a large number of PCB congeners into meaningful analytic units. METHODS: In a case-control study of serum PCB levels on breast cancer risk, measured levels of 56 PCB congener peaks were available for analysis.

We considered several approaches for grouping these compounds based on 1) chlorination, 2) factor analysis, 3) enzyme induction, 4) enzyme induction and occurrence, and 5) enzyme induction, occurrence, and other toxicological aspects. The utility of a framework was based on the mechanism of biologic actions within each framework, lack of collinearity among congener groups, and frequency of detection of PCB congener groups in measured serum levels of 192 healthy postmenopausal women. RESULTS: Most participants had detectable levels for the proposed PCB congeners groups, using degree of chlorination as a grouping framework. In addition, the previously proposed grouping approach based on enzyme induction, occurrence, and other toxicological aspects was an applicable alternative to the crude approach of grouping by degree of chlorination. Grouping these congeners with respect to P450 enzyme induction activity, and the previously proposed framework based on enzyme induction and occurrence, did not fit these data as well, because only a small proportion of participants had detectable levels for the congener groups with the greatest toxicological potential. Statistical grouping did not result in an interpretable and meaningful clustering of these exposures. CONCLUSIONS: In these data, grouping with respect to degree of chlorination and the previously proposed framework based on enzyme induction, occurrence, and other toxicological aspects were the most useful approaches to reducing a large number of PCB congeners into meaningful analytic units. Factors affecting the utility of the proposed grouping frameworks are discussed.

Niewiadomy A, Matysiak J, Zabinska A, Rozylo JK, Senczyna B, Jozwiak K. **Reversed-phase high-performance liquid chromatography in quantitative structure-activity relationship studies of new fungicides.** J Chromatogr A 1998;828(1-2):431-8.

Richard AM. **Commercial toxicology prediction systems: a regulatory perspective.** Toxicol Lett 1998;102-103:611-6.

The use of commercial toxicity prediction systems in a regulatory setting must consider both the limitations and capabilities of the methods, as well as the ultimate use of the predictions, e.g. for testing prioritization, screening, or supporting regulatory decisions. Current systems are better suited to hazard identification (i.e. positive identification of activity-conferring features) than to ruling out hazard. Two recent examples (an EPA testing prioritization exercise for water disinfection byproducts and a regulatory action on 2,4,6-tribromophenol) illustrate issues involved in regulatory applications of SAR and commercial prediction systems. The challenge for the future will be to improve technologies for prediction within the constraints of available data, make optimal use of new test data, and better integrate elements of quantitative modeling (QSAR), empirical association, and biological and chemical mechanisms towards the goal of toxicity prediction.

Rozylo JK, Niewiadomy A, Zabinska A, Matysiak J. **RPTLC investigation of the hydrophobicity and biological activity of new fungicidal compounds.** J Planar Chromatogr Modern TLC 1998;11(6):450-6.
BIOSIS COPYRIGHT: BIOL ABS. Reversed-phase thin-layer chromatography (RPTLC) has been used to evaluate the hydrophobicity and antimycotic activity of dihydroxythiobenzanilides, newly synthesized bioactive compounds with fungicidal properties. The retention behavior of the compounds has been examined with water-acetone or water-methanol as mobile phases and the linear relationship between

the volume fraction of the organic modifier and the logarithm of the capacity factor was established for every solute over a limited range. It was shown that the theoretical capacity factor obtained by extrapolation to pure aqueous mobile phase of retention data for the water-organic modifier systems was suitable for quantitative description of the hydrophobicity of the solutes in a way closely related to the lipophilicity Hansch parameters. Deviations from this relationship were found for compounds with substituents which participate in strong intramolecular interactions. The equation describing the structure-activity relationship (QSAR) indicated the importance of the hydrophobic character and the structure of substituents in determining the antimycotic activity of the compounds. The examined dependencies were more statistically significant for acetone-water systems than for those employing methanol-water, thus implying the greater suitability of acetone as organic modifier in QSAR studies of the investigated compounds.

Sinks GD, Carver TA, Schultz TW. **Structure-toxicity relationships for aminoalkanols: a comparison with alkanols and alkanamines.** SAR QSAR Environ Res 1998;9(3-4):217-28.
The relative toxicity (log IGC50(-1)) of 49 selected aliphatic amines and aminoalkanols was evaluated in the static Tetrahymena pyriformis population growth impairment assay. Excess toxicity, indicated by potency greater than predicted for non-polar narcotic alkanols, was associated with both classes of test chemicals. Moreover, the aminoalkanols were found to be more toxic than the corresponding alkanamines. A high quality 1-octanol/water partition coefficient (log K(ow)) dependent quantitative structure-activity relationship (QSAR), logIGC50(-1) = 0.78 (log K(ow)) - 1.42; r2 = 0.934, was developed for alkanamines. This QSAR represented the amine narcosis mechanism of toxic action. No quality QSAR was developed for the aminoalkanols. However, several structure-toxicity features were observed for this class of chemicals. Two-amino-1-hydroxy derivatives being more toxic than the corresponding derivatives, where the amino and hydroxy moieties were separated by methylene groups. Hydrocarbon branching next to the amino moiety resulted in decreased toxicity. Aminoalkanol alters lipid metabolism in T. pyriformis.

Van Der Burght AS, Clijsters PJ, Horbach GJ, Andersson PL, Tysklind M, Van Den Berg M. **Structure-dependent induction of CYP1A by polychlorinated biphenyls in hepatocytes of cynomolgus monkeys (Macaca fascicularis).** Toxicol Appl Pharmacol 1999;155(1):13-23.
Until now structure-activity relationships (SARs) for in vitro or in vivo CYP1A induction by polychlorinated biphenyls (PCBs) have only been determined in rodents and birds. This study describes the first development of such a SAR in a primate species by using hepatocyte cultures of cynomolgus monkey (Macaca fascicularis). Hepatocyte cultures of primate species might be a more suitable model for humans than those of rodents. For 20 PCBs, the in vitro induction of CYP1A activity was determined by measuring dealkylation of either methoxyresorufin or ethoxyresorufin. Selection of PCBs was based on multivariate physical-chemical characterization of all tetra- through heptachlorinated congeners. The non-ortho-substituted congeners were found to be the most potent inducers, followed by the mono-ortho-substituted PCBs. Multiple-ortho-substituted congeners, with more than five chlorine atoms, were inducers of CYP1A activity in monkey hepatocytes as well, with EC50 values approximately 10,000 times higher than 3,3',4,4',5 PeCB (PCB 126), the most potent congener. Using partial least-squares (PLS) modeling, predictions of CYP1A activity were established for all other tetra- to hepta-substituted congeners. Several congeners, which are abundant in the (a)biotic environment, were predicted to have

CYP1A activity in cynomolgus monkey hepatocytes. Because induction of CYP1A activity is generally used as an early and sensitive biomarker for the Ah-receptor-mediated potential of a chemical, further studies are recommended to determine the possible risks of these multiple-ortho PCBs to humans. Copyright 1999 Academic Press.

Yamamoto T, Hori M, Watanabe I, Tsutsui H, Harada K, Ikeda S, Maruo J, Morita T, Ohtaka H. **Synthesis and quantitative structure-activity relationships of N-(3-oxo-3,4-dihydro-2H-benzo[1,4] oxazine-6-carbonyl)guanidines as Na/H exchange inhibitors.** Chem Pharm Bull (Tokyo) 1998;46 (11):1716-23.
N-(3-Oxo-3,4-dihydro-2H-benzo[1,4]oxazine-6-carbonyl)guanidines 4 were prepared and tested for Na/H exchange inhibitory activities in order to clarify the structure-activity relationship (SAR). Quantitative SAR (QSAR) analysis of 6-carbonylguanidines 4 indicated that the length of the 4-substituent was parabolically related to activity and that the calculated optimum 4-substituents were propyl, ethyl and isopropyl groups. This SAR was similar to the SAR of the 2- and 4-substituents of 7-carbonylguanidine derivatives 3, although the position relative to the essential guanidinocarbonyl group was different. Larger 2-substituents, such as a phenyl group were unfavorable. The most potent derivative in this series was N-(4-isopropyl-2,2-dimethyl-3-oxo-3,4-dihydro- 2H-benzo[1,4]oxazine-6-carbonyl)guanidine 4 g, with an IC50 value of 0.12 microM. The methanesulfonate salt (KB-R9032) of 4g had excellent water-solubility and showed anti-arrhythmia activity against a rat acute myocardial infarction model. KB-R9032 was selected for further investigation as a therapy for ischemia-reperfusion induced injury.

Zhu X, Zhang YP, Klopman G, Rosenkranz HS. **Thalidomide and metabolites: indications of the absence of 'genotoxic' carcinogenic potentials.** Mutat Res 1999;425(1):153-67.
Because of the reintroduction into human therapeutics of thalidomide, a recognized developmental toxicant in humans, there has been concern about its potential for inducing other health effects as well. The present study is concerned with the possible mutagenicity and carcinogenicity of this chemical. Using the expert system, META, a series of putative metabolites of thalidomide was generated. In addition to the known or hypothesized metabolites of thalidomide (N=12), a number of additional putative metabolites (N=131) were identified by META. The structures of these chemicals were subjected to structure-activity analyses using predictive CASE/MULTICASE models of developmental toxicity, rodent carcinogenicity and mutagenicity in Salmonella. While thalidomide and some of its putative metabolites were predicted to be developmental toxicants, none of them were predicted to be rodent carcinogens. Putative metabolites containing the hydroxamic acid or hydroxylamine moieties were predicted to be mutagens. None of the 'known' metabolites of thalidomide contained these reactive moieties. Whether such intermediates are indeed generated or whether they are generated and are either unstable in the presence of oxygen or react rapidly with nucleophiles is unknown. Copyright 1999 Elsevier Science B.V.

REPRODUCTIVE AND DEVELOPMENTAL TOXCITY

Binkova B, Vesely D, Vesela D, Jelinek R, Sram RJ. **Genotoxicity and embryotoxicity of urban air particulate matter collected during winter and summer period in two different districts of the Czech Republic.** Mutat Res 1999;440(1):45-58.

This study is the in vitro part of a long-term program to investigate the impact of air pollution on the health of a population in a polluted region of Northern Bohemia. In order to assess the possible health risks associated with a complex mixture of hundreds of organic compounds adsorbed to air particles, we used a biomarker-directed fractionation procedure to evaluate biological activities of different chemical compound classes. The extractable organic compounds from the air particles collected in both the polluted and the control districts during the summers and winters of 1993-1994 were investigated. The principal aim of this study was to compare the DNA binding activities of those compound classes using an in vitro acellular assay coupled with 32P-postlabeling and an embryotoxicity assay using Chick Embryotoxicity Screening Test (CHEST). In both assays, the highest activity was due to the neutral fractions from which the aromatic subfractions containing mainly polycyclic aromatic hydrocarbons (PAHs) and their methyl-derivates were the most active for both localities and seasons. A good correlation between the levels of DNA adduct formation using S9 metabolic activation and the ED50 for all different complex mixtures of organic compounds was observed ($r=0.773$, $p<0.001$). DNA adduct maps and high performance liquid chromatography (HPLC) profiles were similar for samples from both districts and seasons. The major DNA adducts resulting from the crude extracts were identical to those derived from aromatic fractions. The DNA adducts tentatively identified constituted about 50% of the total adducts formed by the crude extracts following S9-metabolic activation. Our results confirmed the similarities of the major ubiquitous emission sources of organic compounds in both districts. This is the first report in which the biological activities of complex mixtures in short-term assays with remarkably different endpoints such as DNA adduct formation and embryotoxicity have been compared. Copyright 1999 Elsevier Science B.V.

Burkhart JG, Helgen JC, Fort DJ, Gallagher K, Bowers D, Propst TL, Gernes M, Magner J, Shelby MD, Lucier G. **Induction of mortality and malformation in Xenopus laevis embryos by water sources associated with field frog deformities [see comments]**. Environ Health Perspect 1998;106(12):841-8. Water samples from several ponds in Minnesota were evaluated for their capacity to induce malformations in embryos of Xenopus laevis. The FETAX assay was used to assess the occurrence of malformations following a 96-hr period of exposure to water samples. These studies were conducted following reports of high incidences of malformation in natural populations of frogs in Minnesota wetlands. The purpose of these studies was to determine if a biologically active agent(s) was present in the waters and could be detected using the FETAX assay. Water samples from ponds with high incidences of frog malformations (affected sites), along with water samples from ponds with unaffected frog populations (reference sites), were studied. Initial experiments clearly showed that water from affected sites induced mortality and malformation in Xenopus embryos, while water from reference sites had little or no effect. Induction of malformation was dose dependent and highly reproducible, both with stored samples and with samples taken at different times throughout the summer. The biological activity of the samples was reduced or eliminated when samples were passed through activated carbon. Limited evidence from these samples indicates that the causal factor(s) is not an infectious organism nor are ion concentrations or metals responsible for the effects observed. Results do indicate that the water matrix has a significant effect on the severity of toxicity. Based on the FETAX results and the occurrence of frog malformations observed in the field, these studies suggest that water in the affected sites contains one or more unknown agents that induce developmental abnormalities in Xenopus. These same factors may contribute to the increased incidence of malformation in native species.

Chen WY, Yang JG, Huang SH, Li PS. **Effects of cyclophosphamide on maturation and subsequent fertilizing capacity of pig oocytes in vitro.** Chin J Physiol 1998;41(2):75-83.
This study examines the effects of cyclophosphamide, a widely used anti-cancer agent, on the maturation of pig oocytes and on their subsequent fertilizing capacity in vitro. Pig cumulus-oocyte complexes collected from prepubertal gilts were cultured in Waymouth MB 752/1 medium supplemented with sodium pyruvate (50 micrograms/ml), luteinizing hormone (0.5 microgram/ml), follicle-stimulating hormone (0.5 microgram/ml), and 17 beta-estradiol (1 microgram/ml) in the presence or absence of cyclophosphamide for 24 hr; they then were cultured without hormonal supplements in the presence or absence of cyclophosphamide for an additional 16-24 hr. The breakdown of germinal vesicle (GVBD) and changes in glutathione (GSH) content before in vitro fertilization were assessed. Oocytes containing one polar body and a metaphase plate were regarded as matured. Cytoplasmic maturation as determined by male pronuclear formation following fertilization in vitro was also examined. Treatment of oocytes with increasing concentrations (1-1000 micrograms/ml) of cyclophosphamide for 48 hr resulted in a dose-response inhibition of the rate of maturation, but had no effect on GVBD. Increasing duration (12-48 hr) of treatment with cyclophosphamide (100 micrograms/ml) led to a time-dependent inhibition of nuclear maturation, achieving statistical significance by 24 hr. The addition of cyclophosphamide (100 micrograms/ml) to maturation medium immediately after culture, 12 hr or 24 hr after culture also decreased the percentage of oocytes matured during a 48-h culture period. Exposure of oocytes to cyclophosphamide (100 micrograms/ml) for 40 hr did not prevent sperm penetration, not affect the incidence of polyspermy, or decrease the ability of oocytes to form a male pronucleus at 8 hr after insemination. The concentration of GSH, an important factor for male pronuclear formation, in pig oocytes was determined by an enzymatic cycling assay. The concentration found was 8.15 +/- 1.19 mM per oocyte. Exposure of oocytes to cyclophosphamide (100 micrograms/ml) had no effect on GSH concentration. These results demonstrate that cyclophosphamide directly inhibits the meiotic but not cytoplasmic maturation of pig oocytes in vitro. This inhibitory effect, apparently, is not mediated through a decrease in the level of intracellular glutathione.

Fort DJ, Propst TL, Stover EL, Strong PL, Murray FJ. **Adverse reproductive and developmental effects in Xenopus from insufficient boron.** Biol Trace Elem Res 1998;66(1-3):237-59.
Frog embryo teratogenesis assay--Xenopus (FETAX) was utilized as a model system to evaluate the effects on embryo-larval development at various low boron (B) exposure levels in the culture media. Concentrations tested ranged from < 1 to 5000 microg B/L. A statistically significant ($P < 0.05$) increase in malformations was observed at < or = 3 microg B/L, but not at the greater concentrations. Abnormal development of the gut, craniofacial region and eye, visceral edema, and kinking of the tail musculature (abnormal myotome development) and notochord were observed. In subsequent studies, adult frogs were maintained for 28 d on two diets: (1) low B (LB, 62 microg B/kg) or (2) boric acid supplemented (BA, 1851 microg B/kg); the frogs were subsequently mated, and their offspring were cultured in media containing various levels of B. Results of the 28-d depletion studies indicated that frogs maintained under LB conditions produced a greater proportion of (1) necrotic eggs and (2) fertilized embryos, which abnormally gastrulated at a greater rate and were substantially less viable than embryos from frogs fed the BA diet. Malformations similar to those seen in the initial study were observed in embryos from the B-depleted adults maintained in an LB environment; 28 d on the LB diet enhanced the

incidence of malformations associated with the LB culture media. These abnormalities were not observed in embryos cultured in > or = 4 microg B/L from adults cultured on the BA diet. These studies showed that insufficient B reproducibly interfered with normal Xenopus laevis development during organogenesis, substantially impaired normal reproductive function in adult frogs, and thus represent the first studies demonstrating the nutritional essentiality of B in an amphibian species.

Haines G, Marples B, Daniel P, Morris I. **DNA damage in human and mouse spermatozoa after in vitro-irradiation assessed by the comet assay.** Adv Exp Med Biol 1998;444:79-91, Discussion 92-3.
The comet assay is widely employed as a method to measure DNA damage in a wide variety of cell types following genotoxic insult. We have used this method in order to characterise DNA damage in spermatozoa following in vitro irradiation with 137Cs gamma rays. In contrast to somatic cells, the DNA of mammalian spermatozoa is bound by protamine molecules allowing a sixfold more highly compact structure and thus rendering conventional cell lysis protocols ineffective. Therefore, this new method uses an extensive lysis step to ensure effective removal of DNA-associated proteins allowing DNA damage to be scored reproducibly in both murine and human spermatozoa. Mouse spermatozoa collected from the vas deferens at post-mortem or human spermatozoa provided by donors were irradiated with doses of gamma-rays from 0-100 Gy using a 137Cs source and then processed for both alkaline and neutral comet assays. Under neutral electrophoresis conditions, which permits the measurement of double-stranded DNA breaks, a linear increase in the amount of DNA damage measured was observed with increasing radiation dose for both murine and human spermatozoa. Similarly, using alkaline electrophoresis conditions to examine DNA single-strand breaks and alkali-labile sites, a linear relationship was also observed for murine sperm but in contrast no such relationship was apparent for human spermatozoa subjected to the same radiation treatments. Interestingly, unirradiated sperm (both human and mouse) showed extensive DNA migration from the nucleus after alkaline assay. Since it is unlikely that the DNA of normal spermatozoa contains high numbers of single-strand breaks and damage was not detected for unirradiated sperm in the neutral assay, it is more likely that this DNA migration is due to the presence of high numbers of alkali labile sites within sperm DNA and that these may be related to the highly condensed structure of spermatozoal DNA. The large radiation doses used in these experiments to produce measurable amounts of DNA damage reflects the high radioresistance of spermatozoa compared to somatic cells and this may also be related to the differences in DNA packaging and conformation. In conclusion, this work shows that the comet assay represents a new method for examining DNA damage in spermatozoa and should be evaluated for use in reproductive toxicity testing.

Hamm JT, Wilson BW, Hinton DE. **Organophosphate-induced acetylcholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (Oryzias latipes).** Neurotoxicology 1998;19 (6):853-69.
Recent monitoring of the Sacramento-San Joaquin River system (CA) indicates that levels of the organophosphate pesticide, diazinon, exceed National Academy of Science guidelines and these levels result in toxicity in USEPA acute toxicity tests with Ceriodaphnia dubia. Since organophosphates (OPs) inhibit acetylcholinesterase (AChE), the present study examined the effects of diazinon on the embryonic nervous system of a model teleost, medaka, Oryzias latipes. Preliminary histological screens revealed limited retinal cell necrosis in control embryos with apparent increased necrosis in diazinon-

exposed embryos. Subsequently, embryos were exposed to 1.8 x 10(-5), 4.4 x 10(-5), or to 8.8 x 10(-5) M diazinon and replicates were frozen for biochemical analysis or were fixed for histopathological analysis at days 3, 5, and 7 of development. Diazinon exposure significantly inhibited AChE activity within whole embryos and in homogenates of retinas from treated animals. Histological examination of embryos indicated that as the retina underwent differentiation into distinct cell layers, between days 5 and 7, small foci of necrotic cells became apparent within the inner nuclear layer and isolated individual pyknotic cells were observed in the ganglion layer. Quantification of foci of necrotic cells revealed that 8.8 x 10(-5) M diazinon increased number and area of these lesions. Enzyme histochemistry localized AChE activity to regions equivalent to sites of necrosis. Separate exposures of embryos to the OP, diisopropylphosphorofluoridate, produced large foci of necrotic cells at sites equivalent to those seen following diazinon exposure.

Herkovits J, Helguero LA. **Copper toxicity and copper-zinc interactions in amphibian embryos.** Sci Total Environ 1998;221(1):1-10.
BIOSIS COPYRIGHT: BIOL ABS. The copper hazard was evaluated by means of a 7-day toxicity test with Bufo arenarum embryos. The LC50 and LC10 values from 24 to 168 h of exposure were approx. 0.085 and 0.05 mg Cu2+/l, respectively, while the LC90 resulted in 0.155 mg Cu2+/l but in this case from 96 h onwards the LC90 diminished up to approx. 0.105 mg Cu2+/l. These data plotted as Toxicity Profiles (TOP) provide a better understanding of concentration and time-dependent thresholds. For instance, exposure threshold occurs within the first 24 h of treatment while for concentration thresholds LC10 and LC90 seem to be more meaningful than LC50 because the S.D. of this last value is overlapping those of LC10 and LC90 for most of the exposure period evaluated. Toxicity data corresponds to a pH of 6.8 which is normal for the maintaining media. Combined treatments of copper and zinc point out a beneficial effect of zinc proportional to the zinc concentration in the maintaining media, e.g. 100% of protection was achieved with 30 mg Zn2+/l for a copper concentration exerting 90% of mortality. The presence of Cu2+ did not enhance Zn2+ toxicity The results are discussed in terms of water quality criteria for wildlife and human health protection purposes.

Hornung MW, Spitsbergen JM, Peterson RE. **2,3,7,8-Tetrachlorodibenzo-p-dioxin alters cardiovascular and craniofacial development and function in sac fry of rainbow trout (Oncorhynchus mykiss).** Toxicol Sci 1999;47(1):40-51.
Hallmark signs of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in rainbow trout sac fry, are yolk sac edema, hemorrhage, craniofacial malformation, and growth retardation culminating in mortality. Our objective was to determine the role of cardiovascular dysfunction in the development of this toxicity. An embryotoxic TCDD dose (385 pg/g egg) caused a progressive reduction in blood flow in rainbow trout sac fry manifested first and most dramatically in the 1st and 2nd branchial arches and vessels perfusing the lower jaw. Blood flow was reduced later in the infraorbital artery and occipital vein of the head as well as segmental vessels and caudal vein of the trunk. Reduced perfusion occurred last in gill branchial arteries involved with oxygen uptake and the subintestinal vein and vitelline vein involved with nutrient uptake. Although heart rate throughout sac fry development was not affected, heart size at 50 days post-fertilization (dpf) was reduced far more than body weight or length, suggesting that the progressive circulatory failure caused by TCDD is associated with reduced cardiac output. Craniofacial development was arrested near hatch, giving rise to craniofacial malformations in which the jaws and

anterior nasal structures were underdeveloped. Unlike the medaka embryo, in which TCDD causes apoptosis in the medial yolk vein, endothelial cell death was not observed in rainbow trout sac fry. These findings suggest a primary role for arrested heart development and reduced perfusion of tissues with blood in the early-life stage toxicity of TCDD in trout.

Kamijo T, Rajabi MR, Mizunuma H, Ibuki Y. **Biochemical evidence for autocrine/paracrine regulation of apoptosis in cultured uterine epithelial cells during mouse embryo implantation in vitro.** Mol Hum Reprod 1998;4(10):990-8.
During embryo implantation, apoptosis is observed morphologically at the implantation site of endometrium. The objectives of this study were to demonstrate biochemical evidence of apoptosis and quantitative assessment of DNA fragmentation in uterine epithelial cells using a mouse implantation model, and to investigate the autocrine/paracrine regulation of apoptosis in uterine epithelial cells during blastocyst outgrowth. Blastocysts from day 4 pregnant mice were cultured on uterine epithelial cells for 96 h. Uterine epithelial cells dislodged by trophoblasts in endometrium-trophoblast unit demonstrated morphological features of apoptosis by Acridine Orange staining. Electrophoresis demonstrated DNA ladder and DNA fragmentation by enzyme-linked immunosorbent assay markedly increased after 48 h period of incubation. Apoptosis increased in an exponential way in accordance with trophoblast outgrowth. In addition, DNA fragmentation was shown in the epithelial cells by adding embryo-conditioned medium (CM) and the effect of embryo CM on apoptosis was significantly inhibited by anti-transforming growth factor (TGF)-beta antibody. Delayed outgrowth was observed after 48 h of incubation in the blastocysts cultured with anti-TGF-beta antibody. These results suggest there is autocrine/paracrine regulation of apoptosis in uterine epithelial cells at mouse embryo implantation and that TGF-beta might play an important role in the occurrence of apoptosis in the endometrium-trophoblast unit.

Kimmel GL. **Invited perspective: in vitro testing in developmental toxicity risk assessment [editorial]**. Teratology 1998;58(2):25-6.

Kloas W, Lutz I, Einspanier R. **Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo.** Sci Total Environ 1999;225(1-2):59-68.
Several environmental chemicals are known to have estrogenic activity by interacting with development and functions of endocrine systems in nearly all classes of vertebrates. In order to get a better insight of potential estrogenic effects on amphibians caused by environmental pollution this study aims to develop a model for investigating endocrine disruptors using the amphibian Xenopus laevis. In that model the potential estrogenic activity of endocrine disruptors is determined at several levels of investigation: (I) binding to liver estrogen receptor; (II) estrogenicity in vitro by inducing vitellogenin synthesis in primary cultured hepatocytes; and (III) in vivo effects on sexual development. Here we deal with establishing methods to assay estrogenic activity of environmental chemicals in vitro and in vivo. In vitro we used a semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) technique to determine mRNA-induction of the estrogenic biomarker vitellogenin in primary cultured hepatocytes of male Xenopus laevis. Time courses of vitellogenin-mRNA in the presence and absence of 10(-6) M 17 beta-estradiol (E2) resulted in a marked loss of mRNA from controls after 2 days while E2 treatment kept vitellogenin-mRNA at a relatively stable level. After 36 h of incubation estrogenic activities of E2,

4-nonylphenol (NP), and 2,2-bis-(4-hydroxyphenyl)-propan (bisphenol A) at concentrations ranging from 10(-10) to 10(-5) M were assayed by RT-PCR of vitellogenin-mRNA and showed the following ranking of dose-dependent potency: E2 > NP > bisphenol A. These in vitro results were confirmed further by in vivo experiments determining sexual differentiation of Xenopus laevis after exposure to E2 and environmental chemicals during larval development. Concentrations of 10(-7) and 10(-8) M E2 as well as 10(-7) M of NP or bisphenol A caused a significant higher number of female phenotypes compared to controls indicating a similar ranking of estrogenic potencies in vivo as in vitro. In addition, butylhydroxyanisol and octylphenol, both showed feminization at 10(-7) M while octylphenol was also effective at 10(-8) M. In summary these results demonstrate for the first time the use of a semiquantitative RT-PCR technique for screening estrogenicity by assaying mRNA induction of the estrogenic biomarker vitellogenin in vitro. The combination of this newly developed method with classical exposure experiments is necessary for determination of the biological significance of estrogenic chemicals.

Krogenaes AK, Nafstad I, Skare JU, Farstad W, Hafne AL. **In vitro reproductive toxicity of polychlorinated biphenyl congeners 153 and 126.** Reprod Toxicol 1998;12(6):575-80.
BIOSIS COPYRIGHT: BIOL ABS. This study was conducted to investigate the applicability of an in vitro technique for maturation, fertilization, cleavage, and growth to blastocysts of bovine oocytes to investigate reproductive toxicologic effects. During maturation, the oocytes were exposed to the di-ortho-substituted PCB congener 2,2',4,4',5,5'-CB (PCB 153) in the three concentrations 0.84 ng/mL, 8.4 ng/mL, and 84 ng/mL or to the non-ortho-substituted PCB congener 3,3'4,4',5-CB (PCB 126) in the three concentrations 1.006 pg/mL, 10.06 pg/mL, and 100.6 pg/mL and compared with control groups. PCB 153 had no effect on maturation but resulted in a reduced proportion of oocytes that cleaved at the highest concentration. There were no differences in blastocyst development among groups. PCB 126 resulted in a reduction in maturation percentage at the highest concentration and in blastocyst development at all concentrations. These results demonstrated adverse effects of PCB congeners on bovine oocytes and showed that this system can be used to evaluate toxic effects on oocytes and preimplantation-stage embryos.

Loch-Caruso R. **A mechanistic-based approach for assessing chemical hazards to parturition.** J Womens Health 1999;8(2):235-48.
Evaluations of environmental hazards to pregnancy often overlook the potential for chemicals to disrupt the final event, childbirth. There are relatively few epidemiologic studies on this topic and even fewer toxicologic investigations. Mechanistic-based approaches offset many of the difficulties that are anticipated with intact laboratory animals, such as interspecies variability in the initiating events, and may allow for rapid and relevant assessment of potential chemical hazards. In vitro systems based on knowledge of the cellular events that underlie parturition may, therefore, facilitate investigation of toxicologic aspects of parturition. Nonetheless, limitations of in vitro mechanistic-based approaches exist. Ultimately, the greatest understanding of risk to pregnancy from environmental chemicals is likely to result from the collaborative efforts of laboratory scientists and epidemiologists.

Maranghi F, Macri C, Ricciardi C, Stazi AV, Mantovani A. **Evaluation of the placenta: suggestions for a greater role in developmental toxicology.** Adv Exp Med Biol 1998;444:129-36.

Both in human and in rat, two types of placenta are present: the yolk sac (YS) and the chorioallantoic placenta. Histiotrophy, alpha-fetoprotein synthesis and blood cell formation occur in YS of both species. Besides, the midgut, primordial germ cells and possibly immunological structures originate from the YS tissue. The specialised cells of the chorioallantoic placenta attach the embryo to the uterus and form the vascular connections necessary for the nutrient transport. The placenta redirects maternal endocrine, immune and metabolic functions to conceptus advantage. These complex activities are sensitive to direct toxicity. Indirect effects on the placental functions might be elicited by immunomodulators and endocrine disrupters. Some experimental models could be utilised to identify possible toxic effects on placenta. Among the in vitro models the rodent giant yolk sac culture may be used to study the transport of materials, morphological and/or biochemical alterations and biotransformation activity of the visceral YS epithelium. Other in vitro approaches utilise human derived trophoblastic cells and tissues to investigate implantation and perimplantation toxicology. Besides specific studies, in vivo reproductive toxicity tests could pay more attention to the evaluation of placental tissues. Nowadays, some physiologically based pharmacokinetic models for developmental toxicity are also available to describe the disposition of toxic substances and their metabolites during pregnancy in rodents. Thus, more detailed studies on the embryo-foetal placenta may provide an important tool to understand developmental toxicity mechanisms, with particular regard to embryolethality and delayed development.

Mitchell JJ, Paiva M, Heaton MB. **The antioxidants vitamin E and beta-carotene protect against ethanol-induced neurotoxicity in embryonic rat hippocampal cultures.** Alcohol 1999;17(2):163-8.
Fetal alcohol syndrome is characterized by numerous nervous system anomalies with the developing hippocampus being highly vulnerable. Other conditions can result from maternal ethanol consumption including oxidative stress. Critical antioxidants, such as vitamin E, can be decreased and antioxidative defenses altered. Gestational day 18 rat hippocampal cultures were exposed to ethanol ranging from 400 to 2400 mg/dl (16 h). MTT assays assessed neurotoxicity. Viability was decreased dose dependently. Supplementation with vitamin E or beta-carotene afforded neuroprotection against all ethanol concentrations. Vitamin E completely ameliorated neuronal loss following 400 and 800 mg/dl ethanol. Vitamin E increased survival to 95%, 79%, 66%, and 75% during 1600, 1800, and 2000 and 2400 mg/dl ethanol compared to nonethanol treatment. Vitamin E increased viability by 38%, 23%, 12%, and 29% at 1600, 1800, 2000, and 2400 mg/dl compared to non-vitamin E-supplemented, ethanol treatment. beta-Carotene completely ameliorated cell loss from 400 mg/dl ethanol and increased survival by 18% at 1600 mg/dl and 12% at 2000 mg/dl. This study demonstrates in vitro antioxidative neuroprotection against developmental ethanol exposure and suggests that nutritional therapies incorporating antioxidants may help protect against deleterious fetal effects from maternal alcohol abuse.

Nimrod AC, Benson WH. **Reproduction and development of Japanese medaka following an early life state exposure to xenoestrogens.** Aquatic Toxicol 1998;44(1-2):141-56.
BIOSIS COPYRIGHT: BIOL ABS. Japanese medaka were exposed to environmentally-relevant concentrations of environmental estrogens: nonylphenol (NP, 0.5, 0.8 and 1.9 mug l-1), methoxychlor (MXC, 0.2, 0.6 and 2.3 mug l-1) and estradiol (E2, 0.01, 0.12 and 1.66 mug l-1). Exposure occurred throughout the first month following hatch. E2 survival ratios following the exposure period were significantly altered compared to control groups. Following a month period of 'growout' in dilution water only, sex ratios were measured and reproductive capabilities assessed. No alteration in sex ratios

was observed following treatment with NP or MXC. All three concentrations of E2 were sufficient to produce exclusively female populations. There was no depreciation in reproductive capability in the NP or MXC-treated fish as measured by fecundity, viability of eggs, or hatchability of eggs. E2-treated female fish had a lower fecundity in the highest concentration.

Pitt JA, Carney EW. **Development of a morphologically-based scoring system for postimplantation New Zealand White rabbit embryos.** Teratology 1999;59(2):88-101.
Rodent whole-embryo culture (WEC) systems are well-established, as are several corresponding morphological scoring systems. Recently, WEC techniques for rabbits have been developed, creating the need for a morphological evaluation system in this species. Consequently, we developed a gestational-age-based quantitative morphology evaluation system for rabbit embryos. Detailed descriptions of 21 embryonic structures, as collected from gestational day (gd) 9-13 rabbit embryos, formed the basis for this evaluation system. These descriptions were then developed into specific criteria for assigning numerical scores to quantify the degree of development of each embryonic structure. The overall morphologic score was calculated as the average of the individual structure scores. To make the system as informative as possible, the numerical scale of the scoring system was gestationally age-based (i.e., range of potential scores was 9.0-13.0). The scoring system was then applied in the evaluation of New Zealand White (NZW) rabbit embryos explanted on gd 9 and cultured for 48 hr. Embryos grown in vitro developed normally, but at a slightly slower rate in vitro than in vivo, as evidenced by the lower morphology score (10.4 in vitro, 11.0 in vivo) and measures of growth (somite number, total protein, and head length). This work firmly establishes the normal archetype of embryonic development in the gd 9-13 NZW rabbit and provides an important tool for the advancement of mechanistic studies of rabbit embryos developing both in vivo and in vitro.

Pitt JA, Carney EW. **Evaluation of various toxicants in rabbit whole-embryo culture using a new morphologically-based evaluation system.** Teratology 1999;59(2):102-9.
In an effort to advance the use of whole-embryo culture (WEC) techniques in the rabbit, we recently developed a gestational-age-based quantitative morphologic evaluation system for rabbit embryos. In the current study, we applied this new morphological scoring system to assess the development of rabbit gestational day (gd) 9 embryos exposed for 48 hr in WEC to the teratogens ethanol (EtOH, 154 mM), 6-aminonicotinamide (6AN, 0.15 mM), and methoxyacetic acid (MAA, 5.0 mM), and the nonteratogen penicillin G (PG, 2.0 mM). Each teratogen at the concentration tested markedly inhibited morphological development, as indicated by significantly lower morphologic scores (10.1+/-0.05, EtOH; 10.2+/-0.05, 6AN; and 9.8, MAA) relative to controls (10.6+/-0.04), and resulted in an increased percentage of malformed embryos (53%, EtOH; 57%, 6AN; 90%, MAA; and 3%, control). Embryonic growth, as measured by head length, somite number, and total embryonic protein, was significantly decreased by each teratogen. The abnormalities produced by teratogen exposure, which included brain, somite, and facial defects, were often similar to those produced following in vivo exposure in rabbits and rodents, and/or in vitro exposure in rodents. In contrast to the teratogen exposure groups, PG had no effect on embryo growth parameters, or on malformation rate (6%), although a slight but statistically significant decrease in morphology score (10.5+/-0.03) was noted. Our preliminary studies demonstrate the usefulness of the morphology evaluation system by quantifying graded differences in development, and indicate that rabbit WEC may be a useful adjunct to rodent WEC in gaining insights regarding

differential interspecies sensitivity.

Powlin SS, Cook JC, Novak S, O'Connor JC. **Ex vivo and in vitro testis and ovary explants: utility for identifying steroid biosynthesis inhibitors and comparison to a Tier I screening battery.** Toxicol Sci 1998;46(1):61-74.
Testis and ovary explants have been proposed as in vitro screens for identifying potential inhibitors of steroid biosynthesis. The goals of the current study were to optimize the conditions of the two assays, to characterize these assays using several compounds with well-defined endocrine activity, and to compare the responses from the explant assays with an in vivo male battery currently undergoing validation using the Crl:CD BR rat in order to evaluate their utility as test systems for screening unknown compounds for possible steroid biosynthesis inhibition activity. There were two components to the testis/ovary assays: ex vivo and in vitro. The ex vivo component used testes/ovaries from animals dosed with the test compounds in vivo, and the in vitro component used testes/ovaries from control animals. For the testis assays, decapsulated testis explants (50 mg) were placed into glass scintillation vials, +/-1.0 IU/ml hCG for 3 h in a shaking water bath (34 degrees C). Following the incubation period, medium was removed, centrifuged, and frozen until assayed for hormone concentrations. A similar procedure was used for the ovary explant assay except that each ovary was incubated separately. The testis explants were evaluated using the following compounds: ketoconazole (KETO), a testosterone biosynthesis inhibitor; aminoglutethimide (AG) (only in vitro) and anastrozole (ANA), aromatase inhibitors; finasteride (FIN), a 5alpha-reductase inhibitor; 17beta-estradiol (17beta-E2), an estrogen receptor agonist; flutamide (FLUT), an androgen receptor antagonist; ICI-182,780 (ICI), an estrogen receptor antagonist; haloperidol (HALO), a D2 receptor antagonist; and reserpine (RES), a dopamine depletor. In the ovary assay, AG (only in vitro), ANA, ICI, and HALO (only in vitro) were evaluated. Addition of fetal calf serum to the medium allowed measurement of estradiol (E2) in the testis assay, but production was not inhibited by ANA or AG. In the ovary explant assay, only AG was identified as inhibiting E2 production in vitro. Hence, both the testis and ovary explant assays appear to have limited utility for detecting aromatase inhibitors. Screening of these nine diverse endocrine-active compounds resulted in all of them being identified as altering the endocrine system when assessed by ex vivo and in vitro testis explants. Using only the in vitro assessment with the criteria of steroid biosynthesis inhibition, four of nine compounds were correctly identified in the testis explant assay (17beta-E2, KETO, FLUT, and HALO). The predictability of both the in vitro and ex vivo ovary assay was 50%, suggesting a 50% false positive or negative rate with unknown compounds. However, of the seven compounds assessed to date (17beta-E2, ICI, ANA, KETO, FLUT, HALO, and RES), all were correctly identified using an in vivo male battery, which also has the capability to detect other endocrine activities. Therefore, the testis and ovary explant assay would not be necessary if one were using an in vivo male battery, since this screen would identify steroid biosynthesis inhibitors and would also identify several other endocrine activities. Because of the difficulties in assessing cytotoxicity and the high false positive/negative rates, the ovary and testis explant assays are not useful as routine screening procedures for detecting steroid biosynthesis inhibitors; however, they may have utility in confirming in vivo findings.

Rice DC, Hayward S. **Lack of effect of 3,3'4,4',5-pentachlorobiphenyl (PCB 126) throughout gestation and lactation on multiple fixed interval-fixed ratio and DRL performance in rats.** Neurotoxicol Teratol 1998;20(6):645-50.

There is evidence that polychlorinated biphenyl (PCB) congeners have differential effects on endpoints of neurotoxicity depending on their chemical structure: specifically, that ortho-substituted congeners are neurotoxic while coplanar (dioxin-like) congeners are relatively inactive in producing neurotoxic effects. This study extends research on the effects of developmental exposure to the coplanar congener 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in Long Evans rats. Dams were dosed with 0, 0.25, or 1 microg/kg/day Monday to Friday beginning 5 weeks before and continuing through gestation and lactation. The first 2-week breeding period produced 10, 7, and 13 litters in the three dose groups, respectively, used in behavioral assessment. Breeding females from the control and low-dose group that did not conceive were rebred after 76 days of dosing, producing 6 and 6 litters used in behavioral testing. One female and male from each litter were tested on a multiple fixed interval-fixed ratio schedule of reinforcement beginning at about 200 days of age, followed immediately by performance on a DRL schedule. There were no compelling indications of a treatment-related effect on either schedule. These same rats failed to exhibit PCB-induced impairment on a spatial delayed alternation task performed prior to the current experiments. This regimen of PCB exposure produced reduced weight gain between birth and weaning in Cohort 1, and decreased thyroxine levels and changes in hematology and serum biochemistry parameters in both cohorts. These data provide further evidence for absence of behavioral toxicity as a result of gestational and lactational exposure to a dioxin-like PCB congener.

Salha O, Abusheika N, Sharma V. **Dynamics of human follicular growth and in-vitro oocyte maturation.** Hum Reprod Update 1998;4(6):816-32.
The physiological trigger for meiotic resumption in the human oocyte is the surge of luteinizing hormone, but it can also occur spontaneously if oocytes are released from antral follicles and cultured in vitro. The development of novel techniques for the culture of murine oocytes has raised the possibility of growing human oocytes to maturity in vitro. Such a system could open the door to a number of techniques with revolutionary consequences. It would clearly be of benefit in basic physiological studies of follicular development, as well as being used to test the effect of toxicological substances on oocyte maturation. More significantly, such a system could provide a source of human oocytes for in-vitro fertilization (IVF) where immature or germinal vesicle oocytes are cultured to maturity before being fertilized. If this can be achieved, it might facilitate oocyte cryopreservation, where surplus oocytes are stored, thus avoiding the need for repeated superovulation. A combination of immature oocyte cryopreservation for later maturation and IVF will provide the opportunity to establish oocyte banks and help overcome some of the practical and ethical dilemmas that are currently shadowing the field of reproductive medicine.

Schwaiger J, Negele RD. **Plasma vitellogenin. A blood parameter to evaluate exposure of fish to xenoestrogens.** Acta Veterinaria Brno 1998;67(4):257-64.
BIOSIS COPYRIGHT: BIOL ABS. Besides natural and synthetic estrogens, a variety of industrial chemicals and pesticides are suspected to mimic the natural estrogen 17beta-estradiol, thereby disrupting the animal endocrine system. Based on observations, such as the occurrence of hermaphroditism and feminization of male fish, many investigations focus on fish as indicator organisms for xenoestrogenic compounds within the aquatic environment. Both in vitro assays and in vivo approaches have been developed to evaluate estrogenic effects of these toxicants. The occurrence of the female specific egg yolk precursor protein vitellogenin (Vtg) in the plasma of male fish has widely been used as an indicator

or biomarker of xenoestrogen exposure. This paper briefly reviews potential xenoestrogens known so far, physiological aspects of vitellogenesis, current applications of Vtg plasma levels in fish as a biomarker for estrogenic compounds, and various aspects concerning the possible biological significance of this parameter.

Sussman NB, Mazumdar S, Mattison DR. **Modeling adverse environmental impacts on the reproductive system.** J Womens Health 1999;8(2):217-26.
When priority topics are being established for the study of women's health, it is generally agreed that one important area on which to focus research is reproduction. For example, increasing attention has been directed to environmental exposures that disrupt the endocrine system and alter reproduction. These concerns also suggest the need to give greater attention to the use of animal toxicologic testing to draw inferences about human reproductive risks. Successful reproduction requires multiple simultaneous and sequential processes in both the male and female, and the effect of toxicity on reproduction-related processes is time dependent. Currently, however, the risk assessment approach does not allow for the use of multiple processes or for considering the reproductive process response as a function of time. We discuss several issues in modeling exposure effects on reproductive function for risk assessment and present an overview of approaches for reproductive risk assessment. Recommendations are provided for an effective animal study design for determining reproductive risk that addresses optimization of the duration of dosing, observation of the effects of exposure on validated biomarkers, analysis of several biomarkers for complete characterization of the exposure on the underlying biologic processes, the need for longitudinally observed exposure effects, and a procedure for estimating human reproductive risk from the animal findings. An approach to characterizing reproductive toxicity to estimate the increased fertility risks in a dibromochloropropane (DBCP)-exposed human population is illustrated, using several reproductive biomarkers simultaneously from a longitudinal rabbit inhalation study of DBCP and an interspecies extrapolation method.

Vinggaard AM, Joergensen EC, Larsen JC. **Rapid and sensitive reporter gene assays for detection of antiandrogenic and estrogenic effects of environmental chemicals.** Toxicol Appl Pharmacol 1999;155 (2):150-60.
Reports on increasing incidences in developmental abnormalities of the human male reproductive tract and the recent identifications of environmental chemicals with antiandrogenic activity necessitate the screening of a larger number of compounds in order to get an overview of potential antiandrogenic chemicals present in our environment. Thus, there is a great need for an effective in vitro screening method for (anti)androgenic chemicals. We have developed a rapid, sensitive, and reproducible reporter gene assay for detection of antiandrogenic chemicals. Chinese Hamster Ovary cells were cotransfected with the human androgen receptor expression vector and the mouse mammary tumour virus (MMTV)2-luciferase vector using the new nonliposomal transfection reagent FuGene. Stimulation of the cells for 24 h with the synthetic androgen receptor agonist, R1881 (10 nM), resulted in a 30- to 60-fold induction of luciferase activity. The classical antiandrogenic compounds hydroxy-flutamide, bicalutamide, spironolactone, and cyproterone acetate together with the pesticide(metabolite)s, vinclozolin, p,p'-DDE, and procymidone all potently inhibited the response to 0.1 nM R1881. Compared to the traditional calcium phosphate transfection method, this method has the advantage of being more feasible, as the assay can be scaled down to the microtiter plate format. Furthermore, the transfection reagent is

noncytotoxic, allowing its addition together with the test compounds thereby reducing the hands-on laboratory time. This assay is a powerful tool for the efficient and accurate determination and quantification of the effects of antiandrogens on reporter gene transcription. To extend the application of FuGene, the reagent was shown to be superior compared to Lipofectin for transfecting MCF7 human breast cancer cells with an estrogen response element-luciferase vector. Thus, FuGene may prove to be valuable in diverse reporter gene assays involving transient transfections for screening of potential endocrine disruptors for (anti)androgenic and (anti)estrogenic properties. Copyright 1999 Academic Press.

Young JF. **Physiologically-based pharmacokinetic model for pregnancy as a tool for investigation of developmental mechanisms.** Comput Biol Med 1998;28(4):359-64.
There is no known mechanism of teratogenesis! Although receptor occupancy has been implicated, associated and/or deemed necessary for some malformations in newborns (e.g. estrogen receptors, retinoic acid receptors), the upstream and downstream events from receptor occupancy that definitively tie a xenobiotic exposure with a resultant malformation are essentially unknown. One part of the puzzle that can be delineated is the xenobiotic target-tissue exposure curve. Physiologically-based pharmacokinetic (PBPK) models are designed to provide time-course exposure curves for organs, tissues and fluids of human or animal systems. In this context pregnancy requires special considerations in that the PBPK model must represent the dynamic growth of both the maternal and embryo/fetal systems.

Zhang Benzhong, Wu Desheng. **[Effect of fluoride on proliferation and differentiation in rat and mouse embryo bud cell in vitro]**. J West China U Med Sci 1998;29(3):256-8, 268. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. The effect of fluoride on differentiation and proliferation of rat and mouse embryo limb bud cell were studied with micromass cultures in vitro. Embryo limb bud cells of rat (13-day) and mouse (12-day) were subjected to culture for 5 days. The results showed that fluoride could inhibit differentiation of cells without affecting cells proliferation. The concentrations of 50% inhibition of cell differentiation (IDso) were 6.8mug/ml (rat) and 7.3mug/ml (mouse). The concentrations of 50% inhibitions of cell proliferation (IP50) were 44.1 mug/ml (rat) and 63.6mug/ml (mouse). The IP50/ID50 values 6.4(rat) and 8.7 (mouse) were both greater than 5. According to the assessment criteria of Flint and Cheng Wanrong, the fluoride may be an embryo limb bud cells specific inhibitor. It could have potent teratogenicity.

MISCELLANEOUS

Collan Y. **Alternatives for morphometric and stereologic analysis in toxicopathology.** Toxicol Lett 1998;102-103:393-7.
Total cell numbers within a confined tissue volume, or fractions of cell numbers may be relevant in toxicopathology. They can be estimated with the disector, or the formula of Ebbeson and Tang. For the latter the thicknesses of the sections should bey estimated, e.g. with confocal microscopy, or with a vertically embedded section. The use of these methods, in combination with Cavalieri's principle, will avoid the inconsistencies possibly associated with differences in the level of sectioning. Biochemical tests on intracellular specific molecules may be applied on homogenized tissue. If the tissue is composed of different cell types reacting differently to the tested substance, it may be necessary to estimate the

fractions of different types of cells in the tissue concerned. Because of cell size differences, single sections do not give truthful results. The problem is solved by applying the formula of Ebbeson and Tang.

Feron VJ, Cassee FR, Groten JP. **Toxicology of chemical mixtures: international perspective.** Environ Health Perspect 1998;106(Suppl 6):1281-9.
This paper reviews major activities outside the United States on human health issues related to chemical mixtures. In Europe an international study group on combination effects has been formed and has started by defining synergism and antagonism. Successful research programs in Europe include the development and application of statistically designed experiments combined with multivariate data analysis and modeling in vitro and in vivo studies on a wide variety of chemicals such as petroleum hydrocarbons, aldehydes, food contaminants, industrial solvents, and mycotoxins. Other major activities focus on the development of safety evaluation strategies for mixtures such as the use of toxic equivalence factors or alternatives such as the question-and-answer approach, fractionation followed by recombination of the mixture in combination with a mixture design, and quantitative structure-activity relationship analysis combined with lumping analysis and physiologically based pharmacokinetic/ pharmacodynamic modeling for studying complex mixtures. A scheme for hazard identification and risk assessment of complex mixtures and a consistent way to generate total volatile organic compound values for indoor air have also been developed. Examples of other activities are carcinogenicity studies on complex mixtures (petroleum middle distillates, foundry fumes, pesticides, heterocyclic amines, diesel exhaust, solid particles), neurotoxicity studies of mixtures of solvents alone or in combination with exposure to physical factors, and toxicity studies of outdoor air pollutants, focusing on particulates. Outside the United States, toxicologists and regulators clearly have a growing interest in the toxicology and risk assessment of chemical mixtures.

Morais S, Sousa JP, Fernandes MH, Carvalho GS, De Bruijn JD, Van Blitterswijk CA. **Decreased consumption of Ca and P during in vitro biomineralization and biologically induced deposition of Ni and Cr in presence of stainless steel corrosion products.** J Biomed Mater Res 1998;42(2):199-212.
The purpose of this study was to investigate the effects of 316L stainless steel (SS) corrosion products on the in vitro biomineralization process, because tissue necrosis, bone loss, impaired bone mineralization, and loosening of orthopedic implants are associated with ions and debris resulting from biodegradation. Rat bone marrow cells were cultured in experimental conditions that favored the proliferation and differentiation of osteoblastic cells and were exposed to SS corrosion products obtained by electrochemical means for periods ranging from 1 to 21 days. Quantification of total and ionized Ca and P, as well as Fe, Cr, and Ni, ions in the culture media of control and metal added cultures during the incubation period was performed to study the influence of corrosion products on the Ca and P consumption that occurs during the mineralization process. Control cultures and metal effects on cultures were evaluated concerning DNA content, enzymatic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and alkaline phosphatase (ALP) activity. Histochemical detection of ALP, Ca, and phosphate deposition, and examination of the cultures by scanning and transmission electron microscopy (SEM and TEM) were also performed. The presence of SS corrosion products resulted in impairment of the normal behavior of rat bone marrow cultures. Levels of Cr and Ni in the medium of cultures exposed to 316L SS corrosion products decreased throughout the incubation

period, suggesting a regular deposition of these species; these results were supported by TEM observation of the cultures. Cultures exposed to the corrosion products presented lower DNA content, MTT reduction, and ALP activity and failed to form mineralized areas. These cultures showed negative staining on histochemical reactions for the identification of calcium and phosphate deposition and SEM and TEM examination did not show mineral globular structures or mineralization foci, respectively, which is characteristic of cultures grown in control conditions. These results suggest that metal ions associated with 316L SS are toxic to osteogenic cells, affecting their proliferation and differentiation.

Mumtaz MM, De Rosa CT, Groten J, Feron VJ, Hansen H, Durkin PR. **Estimation of toxicity of chemical mixtures through modeling of chemical interactions.** Environ Health Perspect 1998;106 (Suppl 6):1353-60.
The Agency for Toxic Substances and Disease Registry (ATSDR), in collaboration with the Dutch Organization for Applied Scientific Research (TNO) Nutrition and Food Research Institute, is conducting studies to evaluate the role of chemical interactions in the expression of toxicity from low-level exposure to combinations of chemicals. The goal of this collaborative effort is to use a weight-of-evidence (WOE) approach to estimate joint toxicity of some simple chemical mixtures and to compare the estimations with test results from animal toxicity studies. The WOE approach uses individual chemical dose-response assessments and algorithms that incorporate various assumptions regarding potential chemical interactions. Qualitative evaluations were prepared for binary combinations of chemicals for the effect of butyl hydroxyanisole on di(2-ethylhexyl)phthalate, the effect of stannous chloride on Cd chloride (CdCl2), and the effect of CdCl2 on loperamide. Analyses of these evaluations and their comparison with the conclusions of laboratory animal experiments indicate that the WOE approach can be used to estimate qualitatively the joint toxicity of such simple mixtures. To further test the utility of the WOE approach, qualitative and semiquantitative evaluations were prepared for two chemical mixtures--one with similarly acting halogenated aliphatics (trichloroethylene, tetrachloroethylene, hexachloro-1,3-butadiene[HCBD], and 1,1,2-trichloro-3,3,3-trifluoropropene [TCTFP]) and the other with dissimilarly acting nephrotoxic components (mercuric chloride, lysinolalanine, D-limonene, and HCBD). These two sets of data were used to estimate the overall toxicities of the mixtures using the WOE algorithm for the mixture. The comparison of the results of the estimated toxicity with experimentally determined toxicity of the mixture of similarly acting nephrotoxicants demonstrated that the WOE approach correctly adjusted for the observed interactions in experimental animal studies. However, this was not true for the mixture of dissimilarly acting nephrotoxicants. This could be attributed to the fact that WOE evaluations are based on dose additivity that postulates that all chemicals in a given mixture act in the same way--by the same mechanism--and differ only in their potencies. In these cases the WOE approach evaluations, based on consideration of common mechanisms for simple chemical mixtures, can lead to better estimates of joint toxicity of chemical mixtures than the default assumption of dose additivity. The results also show that the WOE evaluations should be target-organ specific because none of the models tested could approximate the observed responses in organs other than the target organs in the laboratory animal studies.

Oreffo RO, Driessens FC, Planell JA, Triffitt JT. **Effects of novel calcium phosphate cements on human bone marrow fibroblastic cells.** Tissue Eng 1998;4(3):293-303.
The identification and characterization of biocompatible materials that augment bone cell proliferation

and osteogenic activity have important therapeutic implications in skeletal reconstruction and joint replacement. In the present study, we have examined the effects of three biocements, biocement H, calcium-deficient apatite; biocement F, apatite + CaHPO(4); biocement D, carbonated apatite + CaHPO (4) + CaCO(3) and an amorphous calcium phosphate (ACP) proposed as implant fixing materials, on the growth, differentiation, and cell surface interaction of human bone marrow fibroblastic cells. These cells are known to be progenitors of osteoblasts, chondroblasts, adipocytes, myoblasts, and reticulocytes. Alkaline phosphatase enzyme activity, a marker of the osteoblast phenotype, was increased by a factor of two- to sixfold on carbonated apatite, one- to sixfold on apatite and three- to 10-fold on calcium-deficient apatite, over levels observed on plastic. Cell proliferation was significantly reduced. Photomicroscopic examination indicated high biocompatibility with close adhesion of the bone marrow fibroblastic cells to composites D, F, and H. Longer term marrow cultures (15 days) confirmed the stimulation of cell differentiation, as assessed by collagen production, over cell proliferation, of cells grown on carbonated apatite. Enhanced osteoblastic differentiation was observed on a 70% carbonated apatite, which has a composition similar to bone mineral, whereas cell toxicity was observed on cells grown on amorphous calcium phosphate. This in vitro human bone marrow fibroblast culture system provides a simple and effective method for the evaluation of new biomaterials. The development of these novel cements may be of potential use in orthopedic implants.

Payne MA, Craigmill AL, Riviere JE, Baynes RE, Webb AI, Sundlof SF. **The Food Animal Residue Avoidance Databank (FARAD). Past, present and future.** Vet Clin North Am Food Anim Pract 1999;15(1):75-88.
During the last one-and-one-half decades, FARAD has established an unparalleled compilation of residue and pharmacokinetic information for veterinary species. In order to fulfill its mission, FARAD has become as much a research project as an educational one. Pressing problems, such as disease-altered kinetics, minor-species drug use, and industrial contaminants in livestock, require the new methods of analysis FARAD is developing. The data upon which this work is based can be greatly augmented by participation by other nations. In the United States, it was the cooperation of both academic and regulatory organizations that made the success of FARAD possible. Similar international cooperation can facilitate use of the FARAD model in other countries for the economic benefit of all participants, enhancement of food safety, and promotion of animal welfare.

Ponce RA, Bartell SM, Kavanagh TJ, Woods JS, Griffith WC, Lee RC, Takaro TK, Faustman EM. **Uncertainty analysis methods for comparing predictive models and biomarkers: a case study of dietary methyl mercury exposure.** Regul Toxicol Pharmacol 1998;28(2):96-105.
Biologically based markers (biomarkers) are currently used to provide information on exposure, health effects, and individual susceptibility to chemical and radiological wastes. However, the development and validation of biomarkers are expensive and time consuming. To determine whether biomarker development and use offer potential improvements to risk models based on predictive relationships or assumed values, we explore the use of uncertainty analysis applied to exposure models for dietary methyl mercury intake. We compare exposure estimates based on self-reported fish intake and measured fish mercury concentrations with biomarker-based exposure estimates (i.e., hair or blood mercury concentrations) using a published data set covering 1 month of exposure. Such a comparison of exposure model predictions allowed estimation of bias and random error associated with each exposure model.

From these analyses, both bias and random error were found to be important components of uncertainty regarding biomarker-based exposure estimates, while the diary-based exposure estimate was susceptible to bias. Application of the proposed methods to a simple case study demonstrates their utility in estimating the contribution of population variability and measurement error in specific applications of biomarkers to environmental exposure and risk assessment. Such analyses can guide risk analysts and managers in the appropriate validation, use, and interpretation of exposure biomarker information. Copyright 1998 Academic Press.

Schmalz G, Schuster U, Nuetzel K, Schweikl H. **An in vitro pulp chamber with three-dimensional cell cultures.** J Endod 1999;25(1):24-9.
To better simulate the in vivo situation, a three-dimensional fibroblast cell culture was introduced into an in vitro pulp chamber model. The system was evaluated by testing a series of dental filling materials. After a 24-h exposure with (0.3 or 5 ml/h) and without perfusion of the pulp chamber, the tissues were subjected to a routine MTT assay. Zinc phosphate cement, conventional glass ionomer cements, a silicone impression material, and zinc oxide-eugenol did not influence cell viability, compared with untreated controls; but, a light-curing glass ionomer cement significantly reduced cell survival. Perfusion of the chambers did not significantly influence the results, but perfusion conditions of 5 ml/h lead to a general decrease of cell vitality. The three-dimensional cell culture system in an in vitro pulp chamber seems to be a substantial improvement, because zinc oxide-eugenol does not evoke a cellular reaction (as is the case in vivo), and the test system is sensitive enough to detect other toxicants.

Slob W, Pieters MN. **A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: general framework.** Risk Anal 1998;18(6):787-98.
The use of uncertainty factors in the standard method for deriving acceptable intake or exposure limits for humans, such as the Reference Dose (RfD), may be viewed as a conservative method of taking various uncertainties into account. As an obvious alternative, the use of uncertainty distributions instead of uncertainty factors is gaining attention. This paper presents a comprehensive discussion of a general framework that quantifies both the uncertainties in the no-adverse-effect level in the animal (using a benchmark-like approach) and the uncertainties in the various extrapolation steps involved (using uncertainty distributions). This approach results in an uncertainty distribution for the no-adverse-effect level in the sensitive human subpopulation, reflecting the overall scientific uncertainty associated with that level. A lower percentile of this distribution may be regarded as an acceptable exposure limit (e.g., RfD) that takes account of the various uncertainties in a nonconservative fashion. The same methodology may also be used as a tool to derive a distribution for possible human health effects at a given exposure level. We argue that in a probabilistic approach the uncertainty in the estimated no-adverse-effect-level in the animal should be explicitly taken into account. Not only in this source of uncertainty too large to be ignored, it also has repercussions for the quantification of the other uncertainty distributions.

Vallack HW, Bakker DJ, Brandt I, Brostrom-Lunden E, Brouwer A, Bull KR, Gough C, Guardans R, Holoubek I, Jansson B, et al. **Controlling persistent organic pollutants: what next?** Environ Toxicol Pharmacol 1998;6(3):143-75.
BIOSIS COPYRIGHT: BIOL ABS. Within the context of current international initiatives on the control

of persistent organic pollutants (POPs), an overview is given of the scientific knowledge relating to POP sources, emissions, transport, fate and effects. At the regional scale, improvements in mass balance models for well-characterised POPs are resulting in an ability to estimate their environmental concentrations with sufficient accuracy to be of help for some regulatory purposes. The relevance of the parameters used to define POPs within these international initiatives is considered with an emphasis on mechanisms for adding new substances to the initial lists. A tiered approach is proposed for screening the large number of untested chemical substances according to their long-range transport potential, persistence and bioaccumulative potential prior to more detailed risk assessments. The importance of testing candidate POPs for chronic toxicity (i.e., for immunotoxicity, endocrine disruption and carcinogenicity) is emphasized as is a need for the further development of relevant SAR (structure activity relationship) models and in vitro and in vivo tests for these effects. Where there is a high level of uncertainty at the risk assessment stage, decision-makers may have to rely on expert judgement and weight-of-evidence, taking into account the precautionary principle and the views of relevant stake-holders. Close co-operation between the various international initiatives on POPs will be r.