1999 No. 2

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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GENERAL

Balls M, Fentem JH. **The validation and acceptance of alternatives to animal testing.** Toxicol In Vitro 1999;13(4-5):837-46.
BIOSIS COPYRIGHT: BIOL ABS. Validation is the key to the regulatory status of alternative methods. A series of questions are put, to which answers are given, including the following: What is validation? What is meant by "relevance", "reliability" and "purpose"? Why and when is formal validation necessary? What comes before and after a formal validation study? How have validation criteria been defined, and to what extent have they been harmonized internationally? How are validation studies set up, managed and funded? What i ve validation? Is validation helping or hindering the development of in vitro toxicology and the implementation of the 'Three Rs' of Russell & Burch?

Clemedson C, Ekwall B. **Overview of the final MEIC results: I. The in vitro-in vitro evaluation.** Toxicol In Vitro 1999;13(4-5):657-63.
BIOSIS COPYRIGHT: BIOL ABS. In the MEIC study, the first 30 reference chemicals were tested in 82 in vitro toxicity assays while the last 20 chemicals were tested in 67 assays. To increase understanding of the performance of in vitro toxicity tests, these two subsets of results were compared by principal components analyses (PCA) combined with a "random probe" analysis of five key methodological factors, that is, the results from all pairs of methods which were similar in all other respects than the analysed factor were sy his similarity must depend on the high correlation of results from assays with different cell types(mean R2 0.81) and/or different viability endpoints (mean R2 0.85). Main factors contributing to the 20% dissimilarity of results were different exposure times and the use of phylogenetically distant test objects in the non-analogous ecotoxicological assays. As expected, the new analysis of the 61 methods gave roughly similar results as the previous "random probe" analyses of the other two segments.

Combes RD, Earl LK. **BTS special symposium. In vitro toxicology--priorities for the year 2000. University of Surrey, 23 April 1998. Report of a symposium to discuss the BTS working party report on in vitro toxicology.** Hum Exp Toxicol 1999;18(2):126-36.

Dahl JE. **Irritation of dental adhesive agents evaluated by the HET-CAM test.** Toxicol In Vitro 1999;13(2):259-64.
BIOSIS COPYRIGHT: BIOL ABS. The HET-CAM test (hen's egg-chorioallantoic membrane test) was used to evaluate the irritative potential of dental adhesive agents used to improve the clinical quality of resin-based restorations. The dental adhesives were of the multilayer type, represented by either two separate liquids each for prime and bond or one-liquid systems (prime and bond) used for both applications. The chorioallantoic membrane of 9-day-old fertilized eggs was exposed to 15 different brands of dental adhesives consis coagulation of blood vessels in the chorioallantoic membrane, and the mean detection time for coagulation was less than 100 sec for 20 of the proprietary solutions. All prime and bond, nine of 12 primers and six of 19 bonding agents were classified as strong irritants. The remaining primers and four bonding agents were classified as moderate irritants. The remaining bonding

agents were classified as slight irritants (six products) and non-irritants (three products). The results show that adhesi.

Kumari SI, Jamil K. **Biotechnology: an answer to alternatives for animal model testings.** Curr Sci 1999;76(8):1087-90.
BIOSIS COPYRIGHT: BIOL ABS. Following Peter Singer's version of Animal Liberation and the movement of animal rights activists, there has been a resurgence of new and alternate testing procedures for toxicological evaluation. Scientists and researchers have long been involved in the search of alternate testing methods; however, the regulatory toxicologists, FDA and EPA of several countries do not accept in vitro methods as a substitute for safety tests or alternate methods. This is because the new products are for human con.

Misra RB, Joshi PC. **Phototoxicity evaluation Tetrahymena thermophila as an alternative model.** Indian J Exp Biol 1999;37(8):750-7.
BIOSIS COPYRIGHT: BIOL ABS. Interest in utilizing an alternative to animal method for toxicological evaluation has received considerable attention due to cost effectiveness and the ethical issues involving animal experimentation. Alternative methods for phototoxicity evaluation are significant because of growing concern over increasing health effects due to stratospheric ozone depletion resulting in an increasing penetration of ultraviolet light-B radiation (UVB, 290-320 nm) which contributes to activation of chemical and n) react with the membrane proteins of the erythrocyte. However, in vitro test system using protozoaoffers a promising alternative means of phototoxicity evaluation. Our previous studies have demonstrated that synergistic action of photochemically reactive agents and sunlight produces lethal effects to Paramecium but the protozoan has not received serious consideration for use as an alternative model for phototoxicity evaluation. In the present communication we have described the potential appli.

Salem H, Katz SA, editors. **Toxicity Assessment Alternatives: Methods, Issues, Opportunities.** Totowa (NJ): Humana Press Inc; 1999. 262p.
BIOSIS COPYRIGHT: BIOL ABS. RRM BOOK TOXICITY ASSESSMENT METHODS TOXICOLOGY METHODOLOGY ALTERNATIVES TOXICITY TESTING METHODBIOSIS COPYRIGHT: BIOL ABS. This book is a collection of works on the topic of toxicity assessment. The twenty-four individually authored chapters are divided into five parts: Recent Developments on Alternatives; Current Trends and Future Directions; Mechanistically Based Test Methods as Alternatives; Use of Alternatives in Hazard Assessment Initiatives; and Validation, Regulatory Acceptance, and Animal Protection Perspectives. Each chapter contains an introduction, a comprehensive discussion of the topic, a conclusion or l for toxicologists, cosmetic chemists, pharmacologists, life science researchers, and regulatory officers who are interested in learning about alternatives to standard toxicity assessment methods.

CARCINOGENESIS

Amos CI, Xu W, Spitz MR. **Is there a genetic basis for lung cancer susceptibility?** Recent Results Cancer Res 1999;151:3-12.

The major risk factor for lung cancer is exposure to tobacco smoke. Exposure to radon, heavy metals used in smelting, and asbestos also greatly increase risks for lung cancer. However, only about 11% of tobacco smokers ultimately develop lung cancer, suggesting that genetic factors may influence the risk for lung cancer among those who are exposed to carcinogens. Further support for this hypothesis is provided by several epidemiological studies and also from molecular epidemiological studies. Epidemiological studies show approximately 14-fold increased risks for lung cancer among average tobacco smokers and approximately 2.5-fold increased risks attributable to a family history of lung cancer after controlling for tobacco smoke. Segregation analyses suggest that a rare autosomal dominant gene may explain susceptibility to early-onset lung cancer, but these results explain a minority of lung cancer cases, which include a family history. Therefore, more common genetic variants or polymorphisms are hypothesized to affect lung cancer risk. Environmental carcinogenesis resulting from tobacco smoke exposure is a complex process that can involve activation of procarcinogens that lead to adduct formation and subsequent failure of DNA repair, which should normally remove these adducts. Studies comparing DNA repair capacity among newly diagnosed lung cancer patients and age-matched controls indicate significant differences between the two groups. On culturing with bleomycin lymphocytes from lung cancer patients and age- and ethnicity-matched controls, the lymphocytes from lung cancer cases have been consistently observed to show higher levels of chromatid breaks than the control lymphocytes. A similar assay has been developed using benzo-[alpha]pyrene diol-epoxide (BPDE), a reactive substrate that is derived by in vitro processes from benzo[alpha]pyrene, a major carcinogen in tobacco smoke. Results from this assay show an even more significantly higher level of damaged chromatids in lung cancer patients than in controls. Poor DNA repair is independent of tobacco smoking status. The cellular processes involved in DNA repair of bleomycin and BPDE have not yet been fully elaborated. However, the consistency of findings with these two carcinogens indicates that DNA repair capacity influences risk for lung cancer among individuals.

Arbeit JM, Riley RR, Huey B, Porter C, Kelloff G, Lubet R, Ward JM, Pinkel D.
Difluoromethylornithine chemoprevention of epidermal carcinogenesis in K14-HPV16 transgenic mice. Cancer Res 1999;59(15):3610-20.
To be informative for chemoprevention, animal models must both closely emulate human disease and possess surrogate endpoint biomarkers that facilitate rapid drug screening. This study elucidated site-specific histopathological and biochemical surrogate endpoint biomarkers of spontaneous epidermal carcinogenesis in K14-HPV16 transgenic mice and demonstrated that the incidence and severity of these markers were decreased by the ornithine decarboxylase (ODC) inhibitor difluoromethylornithine (DFMO). The cumulative incidence of visible epidermal cancers in 127 untreated transgenic mice was 42% by 52 weeks of age, most frequently affecting the chest as flat lesions in association with chronic ulcers, or in the ear as protuberant masses. Microscopic malignancies were detected in 39% of 32-week-old transgenic mice and were found to emerge from precursor lesions that were of two distinct types: dysplastic sessile ear papillomas and hyperproliferative follicular/interfollicular chest dysplasias. ODC activity and tissue polyamine contents were differentially elevated in ear and chest skin during carcinogenesis, such that there was a marked elevation of both parameters of polyamine metabolism as early as 4 weeks of age in the ear, whereas in the chest, polyamine metabolism was increased significantly only in the late stages of neoplastic progression and in epidermal cancers. Administration of 1.0% DFMO in the drinking water from 4 to 32 weeks of age prevented both visible and microscopic

malignancies and significantly decreased the incidence of chest and ear precursor lesions. ODC activity and tissue putrescine content were markedly diminished by DFMO chemoprevention in ear skin, whereas there was a more modest decline of these parameters in chest skin. DFMO treatment of transgenic mice from 28 to 32 weeks of age was associated with an absence of ear cancer and a marked regression of dysplastic papillomas. In contrast, the results in chest skin were complex in that the severity of chest precursors diminished, but their incidence was unchanged, and microscopic cancers were still detectable within these lesions. Collectively, this study highlights the utility of multistage epidermal carcinogenesis in K14-HPV16 transgenic mice both for the study of the biology of, and as a screening tool for, novel drugs and chemopreventive regimens.

Banasiak D, Barnetson AR, Odell RA, Mameghan H, Russell PJ. **Comparison between the clonogenic, MTT, and SRB assays for determining radiosensitivity in a panel of human bladder cancer cell lines and a ureteral cell line.** Radiat Oncol Investig 1999;7(2):77-85.
Using a series of human bladder cancer cell lines and an immortalised normal ureteral cell line, radiosensitivities measured by three different methods after a single dose of X-radiation are compared. Clear differences between cell survival curves obtained using the clonogenic, microtetrazoline (MTT) and sulforhodamine B (SRB) assays are shown. The most sensitive of the assays investigated was the clonogenic assay. The MTT and SRB assays were found to be relatively insensitive especially at lower radiation levels, suggesting that these assays may not be suitable for predicting therapeutic dose schedules in vivo, but will be important for investigating radio-sensitivity in cell lines with very low plating efficiencies. Each assay discriminated between a range of sensitivities in the cell lines examined, and with some minor differences, the ordering of sensitivities using the three assays was similar. Possible explanations for the differences between results obtained with the three assays are discussed.

Brown-Peterson NJ, Krol RM, Zhu Y, Hawkins WE. **N-nitrosodiethylamine initiation of carcinogenesis in Japanese medaka (Oryzias latipes): hepatocellular proliferation, toxicity, and neoplastic lesions resulting from short term, low level exposure.** Toxicol Sci 1999;50(2):186-94.
BIOSIS COPYRIGHT: BIOL ABS. To investigate relationships among carcinogen exposure, cell proliferation, and carcinogenesis, 14-day post-hatch Japanese medaka (Oryzias latipes) were exposed to 0, 10, 25, 50, or 100 ppm N-nitrosodiethylamine (DEN) for 48 h under static renewal conditions. They were then held in clean water until sampling at 3 and 6 months. The frequencies of hepatic lesions and neoplasms were determined from hematoxylin/eosin-stained paraffin sections. A significant ($p < 0.0001$) concentration-related increas t 6 months. Basophilic foci were significantly related ($p < 0.0001$) to DEN concentration at 3 months post-exposure and were unaffected by gender or age. At both 3 and 6 months, there were significant concentration-related increases in hepatocellular carcinoma (p : 0.02). Hepatocyte proliferation in 3-month whole specimens was quantified using an immunohistochemical assay for proliferating-cell nuclear antigen. Trend tests and a probit dose-response model showed a significantly positive correlati m, and a 6-month sampling period.

Bunton TE. **Use of non-mammalian species in bioassays for carcinogenicity.** IARC Sci Publ 1999; (146):151-84.
The high costs of bioassays for carcinogenicity in rodents have sparked interest in the use of non-mammalian species as possible alternatives. Invertebrate and lower vertebrate species have been used for many years in bioassays for teratogenicity, toxicity and carcinogenicity involving exposure to a

range of genotoxic compounds. Carcinogenicity tests have shown that the development of neoplasia in non-mammalian species is predictable and reproducible and that the results are affected by species, age, chemical class and dose. One disadvantage of using these species in cancer bioassays is the absence of tissues of critical importance in human cancer, such as prostate, lung and breast; however, the similarities between mammals and lower species in basic cellular responses to carcinogens allow reliable correlation of many mechanisms of cancer development down the phylogenetic tree.

Chatterjee S, Hirota H, Belfi CA, Berger SJ, Berger NA. **Hypersensitivity to DNA cross-linking agents associated with up-regulation of glucose-regulated stress protein GRP78.** Cancer Res 1997 Nov 15;57(22):5112-6.
We have shown previously that NAD/poly(ADP-ribose) polymerase-deficient cells that overexpress Mr 78,000 glucose-regulated stress protein (GRP78) are resistant to topoisomerase II inhibitors, such as etoposide, m-amsacrine, and doxorubicin. However, these cells have been found to be hypersensitive to DNA cross-linking agents, including melphalan, cisplatin, and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). These observations prompted us to examine whether overexpression of GRP78 is associated with modulation of cytotoxicity of clinically useful DNA-cross-linking agents such as melphalan, BCNU, and cisplatin. We up-regulated GRP78 in V79 Chinese hamster cells by 2-5-fold using two independent approaches that include exposure to 6-aminonicotinamide, or 2-deoxyglucose. Subsequently, these GRP78-overexpressing cells were trypsinized, plated in regular medium without GRP78-inducing agents, and allowed a 5-h attachment time before being treated with melphalan, BCNU, or cisplatin for 1 h to determine clonogenic survivals. In addition, repair of DNA cross-links induced by those agents were determined by alkaline elution assay. Our results show that the GRP78-overexpressing V79 cells are hypersensitive to DNA cross-linking agents compared to the control V79 cells. Furthermore, repair of drug-induced DNA cross-links appears to be considerably slower in these cells relative to that found in control V79 cells. Thus, our results suggest that (a) up-regulation of GRP78 is associated with an impairment of DNA cross-link repair, (b) up-regulation of GRP78 is associated with potentiation of cytotoxicity induced by alkylating and platinating agents, and (c) up-regulation of GRP78 can be considered as a potentially useful tool to modulate the cytotoxicity of clinically useful alkylating and platinating agents.

Cheng RH, Gant TW. **Detection of tamoxifen-DNA adducts on lacI genes using DNA polymerase stop assay.** Chang Keng I Hsueh Tsa Chih 1999;22(2):189-96.
BACKGROUND: Tamoxifen forms DNA adducts in rat liver and causes an increased mutation frequency at the lacI genes in the livers of lambda/lacI transgenic rats. Although an elevated occurrence of endometrial cancer is found in a small proportion of breast cancer patients treated with tamoxifen, there is conflicting evidence on whether or not low levels of DNA adducts are formed in humans. METHODS: Based on the finding that the progression of DNA/RNA polymerases on templates might be blocked by bulky DNA adducts, we successfully developed and used a polymerase stop assay to map the sites of adduct formation in the target lacI gene following its reaction in vitro with alpha-acetoxytamoxifen and horseradish peroxidase/H2O2 (HRP/H2O2) activated 4-hydroxytamoxifen. RESULTS: Using a T4 DNA polymerase stop assay, adduct formation in the lacI gene of the plasmid constructs, after the reaction in vitro with alpha-acetoxytamoxifen and HRP/H2O2 activated 4-hydroxytamoxifen, was found to mainly occur with guanines. In particular, one site of adenosine

adduction was found on a triplet of adenosines located between two runs of guanines. CONCLUSION: The success of our development of DNA polymerase stop assay to map the sites of tamoxifen-DNA adducts formation will be very useful for the investigation of the mutagenicity/carcinogenicity of tamoxifen. The mutagenic potential of the tamoxifen adducted bases shall be further examined by transfecting the adducted plasmids into suitable human cell lines. Also, further investigations of the sequence specificity in specific oncogenes and tumor suppressor genes may be useful to explore the relationship between the occurrence of human endometrial cancer and tamoxifen treatment.

De Camargo JL, Salvadori D, Rocha NS, Barbisan LF, Ribeiro LR. **The detection of chemical carcinogens in an alternative medium-term bioassay.** Cien Cultur 1999;51(1):22-6.
BIOSIS COPYRIGHT: BIOL ABS. The currently most important available experimental tool to identify chemical carcinogens is the long-term assay with rodents. This is a complex and expensive operational procedure and few, if any, Latin-American countries have enough expertise and/or suitable facilities to run such test. Consequently, these countries are dependent on technical and scientific knowledge generated abroad on carcinogenic hazard identification and on risk analysis and assessment. On the other hand, several disadvant a valid source of evidence of the carcinogenic potential of chemicals. Since then, our laboratory at the Universidade Estadual Paulista (UNESP) has been using this alternative bioassay for testing chemicals. The assays have been performed according to Good Laboratory Procedures policy. Developed on a routine basis, such bioassay protocol may improve the Brazilian expertise on hazard identification and risk assessment and therefore will contribute to a more effective governmental regulatory acti.

Elmore E, Sun C, Li HR, Wyatt GP, Buckmeier JA, Steele VE, Kelloff GJ, Redpath JL. **In vitro chemopreventive efficacy screening using human keratinocytes and the in vivo data correlation.** Anticancer Res 1999;19(2a):909-18.
Agents with potential cancer preventive activity were screened for efficacy in the Human Epidermal Cell (HEC) Assay. The HEC Assay measures inhibition of propane sultone-induced changes in the growth and/or differentiation in early passage keratinocyte cultures. The assay biomarkers were calcium tolerance, growth inhibition, and involucrin induction. The HEC Assay also provides information on the cytotoxicity of the agents following acute and chronic exposure. Agents were evaluated at non-toxic doses in the HEC Assay. The HEC Assay has been used to screen twenty-eight agents for chemopreventive efficacy. A positive response in one or more endpoints of the HEC Assay correlates 100% (16/16) with a positive response in one or more of the animal cancer prevention models (J. Cell. Biochem., 26S:29-53, 1996). The overall sensitivity for predicting efficacy in animals is 84%. The available data suggest that a positive assay response appears to be highly predictive of efficacy in vivo.

Harreus U, Schmezer P, Kuchenmeister F, Maier H. **[Genotoxic effect on human mucous membrane biopsies of the upper aerodigestive tract]**. Laryngorhinootologie 1999;78(4):176-81. (Ger)
BACKGROUND: In numerous epidemiologic studies, environmental and occupational substances such as sodium dichromate (Na2Cr2O7), benzo[a]pyren (B(a)P), and N'nitroso-diethanolamine (NDELA) have been shown to be of potential carcinogenic risk on human epithelial cells in the upper aerodigestive tract. METHODS: Using the alkaline microgel electrophoresis technique (comet assay). mucosal cells isolated from biopsies of the upper aerodigestive tract (nose, paranasal sinuses, mouth, pharynx, larynx, and tonsils) were used to analyze target sites for different genotoxic substances and specific sensitivities

of each donor. The cells were freshly isolated by enzymic digestion. 0.5-1 x 10(6) cells per donor were obtained with viabilities between 80-100%. After in vitro incubation, the cells were subsequently subjected to the single cell microgel electrophoresis assay. Results were evaluated regarding the personal history of each donor, focusing on previous exposure to tobacco, alcohol, and occupational compounds. RESULTS: Na2Cr2O7 induced strong genotoxic damage in the nasal and paranasal sinus epithelia as well as in mucosa cells of the larynx. NDELA caused significant damage in mouth cavity epithelia and showed also to be harmful towards mucosa of pharynx and larynx. B(a)P induced fewer DNA strand breaks in mucosal cells of mouth, pharynx and larynx. Significant differences between individuals were apparent for tissue samples from different donors. The genotoxic damage induced in cells of donors with a history of chronic alcohol consumption was significantly higher than in cells of patients without chronic abuse of alcohol. CONCLUSIONS: The data shows that DNA damage in human epithelial cells of the upper aerodigestive tract induced by environmental and occupational substances can be demonstrated using the microgel electrophoresis technique. The influence of chronic alcohol consumption on the genotoxic effects of substances such as NDELA and B(a)P showed the importance of evaluating preexisting compounding factors.

Kerbel RS. **What is the optimal rodent model for anti-tumor drug testing?** Cancer Metastasis Rev 1998;17(3):301-4.
One of the most serious obstacles facing investigators involved in the development and assessment of new anti-cancer drugs is the failure of preclinical rodent tumor models to predict in a reliable way whether a given drug will have anti-tumor activity and acceptable toxicity in humans. Most previous investigations for assessing drug activity in vivo have utilized rapidly growing non-metastatic transplantable mouse or human tumors injected ectopically in syngeneic or nude mice, respectively. Some of the reasons for the inadequacy of such models are well known and, as a result, there has been a gradual movement toward the use of transgenic oncomouse models for anti-cancer drug testing. It is too early to conclude, one way or the other, whether these will be superior to transplantable tumor models. Moreover, such transgenic models have a number of limitations which are not widely appreciated. It is argued that transplantable tumor models, with various modifications, might be made significantly more predictive than current models, and would thus constitute a more economic alternative to the use of large numbers of transgenic oncomice. These modifications include the use of slower growing and genetically tagged (e.g. LacZ or GFP) tumors which are transplanted initially into orthotopic organ sites. These methods would facilitate the growth and detection of distant microscopic and macroscopic metastases, the response of which to anti-cancer drugs, using 'clinically equivalent doses,' could be evaluated.

Kim HS, Kacew S, Lee BM. **In vitro chemopreventive effects of plant polysaccharides (Aloe barbadensis miller, Lentinus edodes, Ganoderma lucidum and Coriolus versicolor).** Carcinogenesis 1999;20(8):1637-40.
A plant polysaccharide, Aloe gel extract, was reported to have an inhibitory effect on benzo[a]pyrene (B[a]P)-DNA adduct formation in vitro and in vivo. Hence, chemopreventive effects of plant polysaccharides [Aloe barbadensis Miller (APS), Lentinus edodes (LPS), Ganoderma lucidum (GPS) and Coriolus versicolor (CPS)] were compared using in vitro short-term screening methods associated with both initiation and promotion processes in carcinogenesis. In B[a]P-DNA adduct formation, APS (180 micrograms/ml) was the most effective in inhibition of B[a]P binding to DNA in mouse liver cells.

Oxidative DNA damage (by 8-hydroxydeoxyguanosine) was significantly decreased by APS (180 micrograms/ml) and CPS (180 micrograms/ml). In induction of glutathione S-transferase activity, GPS was found to be the most effective among plant polysaccharides. In screening anti-tumor promoting effects, APS (180 micrograms/ml) significantly inhibited phorbol myristic acetate (PMA)-induced ornithine decarboxylase activity in Balb/3T3 cells. In addition, APS significantly inhibited PMA-induced tyrosine kinase activity in human leukemic cells. APS and CPS significantly inhibited superoxide anion formation. These results suggest that some plant polysaccharides produced both anti-genotoxic and anti-tumor promoting activities in in vitro models and, therefore, might be considered as potential agents for cancer chemoprevention.

Moore MA, Tsuda H, Tamano S, Hagiwara A, Imaida K, Shirai T, Ito N. **Marriage of a medium-term liver model to surrogate markers--a practical approach for risk and benefit assessment.** Toxicol Pathol 1999;27(2):237-42.
The need for a reliable medium-term alternative to traditional long-term rodent test protocols for carcinogen risk assessment is pressing given the immense variety of compounds being developed for introduction into the human environment. The established lack of a complete correlation between mutagenicity and carcinogenicity means that recourse must be made to an in vivo model. Optimally, this model should be able to detect not only complete carcinogenic or promoting potential but also any ability to inhibit neoplasia. In order to be effective, it must take into account the available detailed knowledge on mechanisms of action of carcinogens and modulating agents. The Ito model, for which a uniquely comprehensive set of background data has already been accumulated, has a solid scientific basis; this model utilizes quantitative data for glutathione S transferase-positive foci as the preneoplasia-based surrogate end point (PSE). A very practical candidate for routine application, its predictive power, its flexibility, and its capacity to incorporate a range of mechanism-based surrogate end points (MSEs) provide a powerful tool for attainment of the twin goals of detecting carcinogenic agents and identifying promising chemopreventors.

Ohmori K, Miyazaki K, Umeda M. **Detection of tumor promoters by early antigen expression of EB virus in Raji cells using a fluorescence microplate-reader.** Cancer Lett 1998;132(1-2):51-9.
As an in vitro assay for possible tumor promoters, we designed a quantitative immunofluorometric method to detect Epstein-Barr virus early antigen (EBV-EA) expression in Raji cells. In this method, anti-EBV-EA monoclonal antibody, a fluorogenic substrate and a fluorescence microtiterplate-reader were employed. 12-O-Tetradecanoylphorbol-13-acetate (TPA) was shown to be a potent inducer. EBV-EA induction by TPA was related to the activation of protein kinase C and phospholipase A2. The chemicals that reacted positively were okadaic acid, diethylstilbestrol, progesterone, sodium phenobarbital, aldrin and dieldrin. Lithocholic acid, testosterone and DDT were equivocal in the present experiments. Eight other chemicals tested did not react.

Okihiro MS, Hinton DE. **Progression of hepatic neoplasia in medaka (Oryzias latipes) exposed to diethylnitrosamine.** Carcinogenesis 1999;20(6):933-40.
Progression of hepatic neoplasia was assessed in medaka (Oryzias latipes) following aqueous exposure to diethylnitrosamine (DEN). Larvae (2 weeks old) were exposed to 350 or 500 p.p.m. DEN for 48 h, while adults (3-6 months old) were exposed to 50 p.p.m. DEN for 5 weeks. Fish were maintained as long as possible to determine malignant potential of resultant neoplasms. A total of 423 medaka with

106 hepatic neoplasms were examined. There were marked differences in tumor prevalence between exposure groups including: (i) higher prevalence of hepatocellular carcinomas in medaka exposed as adults (100% of hepatocellular tumors in adult-exposed medaka were malignant, while only 51.5% of larval hepatocellular tumors were malignant); (ii) higher prevalence of biliary tumors in medaka exposed as larvae (46.4% of all tumors in larval-exposed medaka were biliary versus 8.1% in adult-exposed fish); (iii) higher prevalence of mixed hepato-biliary carcinomas in adult-exposed medaka (24.3%) compared with those exposed as larvae (3%). In addition, a unique hepatocellular lesion termed 'nodular proliferation' was only observed in adult-exposed medaka. The lesion was characterized by small size (50-300 microm), complete loss of normal tubular architecture and variable megalocytosis. Nodular proliferation was distinct from preneoplastic foci of cellular alteration and may represent microcarcinomas. There was a step-wise increase in mean diameter with age (days post-exposure) from nodular proliferation (174 microm, 17 days) to hepatocellular carcinoma (1856 microm, 62 days) and mixed carcinomas (3209 microm, 93 days) in adult-exposed medaka. Metastasis was observed with 19 neoplasms and tumors with the highest metastatic potential were hepatocellular and mixed carcinomas. The most common form of metastasis was trans-coelomic, followed by direct invasion and distant metastasis, presumably via the vascular route. Differences in tumor prevalence between exposure groups were believed to be the result of length of DEN exposure rather than age of fish at the time of exposure. In larval medaka with brief (48 h) DEN exposure, neoplasms are thought to be the result of dedifferentiation of hepatic cells, with slow progression of foci of cellular alteration to benign and then malignant tumors. In contrast, with adult medaka and prolonged (5 week) DEN exposure, neoplasms are believed to result from initiation of committed stem cells and formation of microcarcinomas ('nodular proliferation'), before progressing to larger hepatocellular and then mixed carcinomas.

Shimada T, Gillam EM, Oda Y, Tsumura F, Sutter TR, Guengerich FP, Inoue K. **Metabolism of benzo [a]pyrene to trans-7,8-dihydroxy-7, 8-dihydrobenzo[a]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase.** Chem Res Toxicol 1999;12(7):623-9.
Recombinant human enzymes expressed in membranes obtained from Escherichia coli transformed with cytochrome P450 (P450) and NADPH-P450 reductase cDNAs were used to identify the human P450 enzymes that are most active in catalyzing the oxidative transformation of benzo[a]pyrene in vitro. Activation of benzo[a]pyrene to genotoxic products that cause induction of umu gene expression in Salmonella typhimurium NM2009 by P450 1A1 and P450 1B1 enzymes was found to be enhanced by inclusion of purified epoxide hydrolase (isolated from rat or human livers) with the reaction mixture. High-performance liquid chromatographic analysis showed that P450 1B1 catalyzed benzo[a]pyrene to trans-7, 8-dihydroxy-7,8-dihydrobenzo[a]pyrene at level of approximately 3 nmol min(-)(1) nmol of P450(-)(1) only when epoxide hydrolase was present and P450 1A1 (with the hydrolase) was able to catalyze benzo[a]pyrene at one-tenth of the activity catalyzed by P450 1B1. Kinetic analysis showed that ratio of V(max) to K(m) for the formation of trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in this assay system was 3.2-fold higher in CYP1B1 than in CYP1A1. Other human P450s (including P450s 1A2, 2E1, and 3A4) were found to have very low or undetectable activities toward the formation of trans-7, 8-dihydroxy-7,8-dihydrobenzo[a]pyrene. A reconstituted system containing purified P450 1B1, rabbit liver NADPH-P450 reductase, and human liver epoxide hydrolase was found to catalyze benzo[a] pyrene to trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene at a rate of 0.86 nmol min(-)(1) nmol of P450 (-)(1); the activities were found to be largely dependent on the presence of sodium cholate in the system.

These results suggest that P450 1B1 is a principal enzyme in catalyzing the oxidation of benzo[a]pyrene to trans-7,8-dihydroxy-7, 8-dihydrobenzo[a]pyrene and that the catalytic functions of P450 1B1 may determine the susceptibilities of individuals to benzo[a]pyrene carcinogenesis.

Stiborova M, Schmeiser HH, Wiessler M, Frei E. **Direct evidence for the formation of deoxyribonucleotide adducts from carcinogenic N-nitroso-N-methylaniline revealed by the 32P-postlabeling technique.** Cancer Lett 1999;138(1-2):61-6.
BIOSIS COPYRIGHT: BIOL ABS. N-Nitroso-N-methylaniline (NMA) is an esophageal carcinogen in the rat. NMA forms a benzenediazonium ion (BDI) during microsomal cytochrome P-450 2B1 (CYP2B1) catalyzed metabolism. Using the nuclease P1-enhanced version of the 32P-postlabeling assay we investigated the formation of adducts by NMA with deoxyadenosine 3'-monophosphate (dAp) and deoxyguanosine 3'-monophosphate (dGp). 32P-postlabeling analysis of dAp and dGp, which were modified by NMA activated with microsomes of rats pretreated wi f dAp and dGp adducts.

Yang D, Zhang M, Du J. **[Inhibitory effect of IGF-II antisense RNA on malignant phenotype of hepatocellular carcinoma]**. Chung Hua Kan Tsang Ping Tsa Chih 1999;7(1):39-41. (Chi)
OBJECTIVE: Inhibitory effect of insulin like growth factor II (IGF-II) antisense RNA on malignant phenotype of hepatic cancer cells was studied. METHODS: A 0.1 kb cDNA of human IGF-II was reversely inserted into a eukaryotic expression vector of pcDNA3 and an IGF-II antisense RNA expression vector of pIGF-II As was obtained. The pIGF-II As was then introduced into human hepatic cancer cell line SMMC-7721. The effect of IGF-II antisense RNA which was expressed by pIGF-II As on SMMC-7721 cell cycles progression was measured with FCM. RESULTS: The coloning formation in anchorage-independent assay of SMMC-7721 cells with pIGF-II As was significantly decreased by comparison with control groups of SMMC-7721 cells and SMMC-7721 cells transfected with pcDNA3 vector alone. FCM analysis showed that the S phase of cell cycles for SMMC-7721 cells with pIGF-II As was increased, but there were no obvious changes in the control groups of SMMC-7721 cells and SMMC-7721 cells with pcDNA3. CONCLUSION: The pIGF-II As of IGF-II antisense RNA acts as an inhibitor on carcinogenesis of the SMMC-7721 cells in vitro.

Yazawa Y, Takagi T, Asakura S, Suzuki K, Kano Y. **Effects of 4-hydroperoxy ifosfamide in combination with other anticancer agents on human cancer cell lines.** J Orthop Sci 1999;4(3):231-7.
Ifosfamide is one of the currently available anticancer agents with a broad spectrum of clinical activity against a variety of tumors. To investigate its optimal combinations, we studied the effect of 4-hydroperoxy ifosfamide (the active form of ifosfamide) in combination with other anticancer agents against two human cancer cell lines, MG-63 (an osteosarcoma cell line) and MOLT-3 cells (a T-cell leukemia cell line). The cells were incubated for 4 days and 3 days, respectively, in the presence of 4-hydroperoxy ifosfamide and the other agent. Cell growth inhibition was determined by MTT assay. The effects of these drug combinations at the concentration producing 50% inhibition (IC50) were analyzed by the isobologram method. 4-Hydroperoxy ifosfamide showed additive effects with bleomycin, cisplatin, cytarabine, doxorubicin, etoposide, 5-fluorouracil, and mitomycin C, while it showed a protective effect with methotrexate in both cell lines. 4-Hydroperoxy ifosfamide showed an additive effect with vincristine in the MG-63 cell line, while it showed a sub-additive effect in the MOLT-3 cell line. No anticancer agents tested showed a supra-additive effect with 4-hydroperoxy ifosfamide. These data suggest that ifosfamide is advantageous for simultaneous administration with a majority of the

anticancer agents we studied. Methotrexate is an inappropriate drug for simultaneous administration with ifosfamide.

CYTOTOXICITY

Abou Hashieh I, Camps J, Dejou J, Franquin JC. **Eugenol diffusion through dentin related to dentin hydraulic conductance.** Dent Mater 1998;14(4):229-36.
OBJECTIVES: The purpose of this study was (1) to find an easy way of evaluating the concentration of eugenol in cell culture fluids; (2) to confirm the relationship between the concentration and the cytotoxicity of eugenol in vitro; (3) to evaluate the cytotoxicity of four temporary eugenol-based filling materials: IRM, super EBA, Kalsogen and zinc oxide-eugenol cement; and (4) to establish a relationship between dentin permeability, eugenol diffusion and cytotoxicity. METHODS: (1) The concentration of eugenol was measured with a spectrofluorimeter; (2) the cell viability of L 929 cells cultivated for 24 h with eugenol-containing medium was evaluated by the MTT assay; (3) after measurement of hydraulic conductance, occlusal cavities in human teeth in vitro were filled with the restorative materials. The cytotoxicity was measured with undiluted test medium and with various dilutions in culture medium; (4) after Lp measurement, the eugenol concentration in the media in the pulp chamber that diffused from IRM and 10(3) mol/l eugenol solution was measured. RESULTS: (1) A proportional relationship ($p = 0.001$ and $r = 1$) was found between the concentration of eugenol; (2) eugenol started to be cytotoxic at 10(-5) mol/l and killed 95% of the cells at 10(-3) mol/l; (3) zinc oxide-eugenol cement was the most cytotoxic filling material when tested with the 1:100 dilution; (4) a significant relationship was found between Lp and cytotoxicity ($p = 0.04$) depending on the dilution of the test medium. A significant relationship was found between Lp and eugenol diffusion from a 10(-3) mol/l solution ($p = 0.03$) but not between Lp and eugenol diffusing from solid IRM (non significant). SIGNIFICANCE: Eugenol diffusion from zinc oxide-eugenol cement appears to depend more on the role of hydrolysis of eugenol from zinc oxide-eugenol cement than on dentin permeability.

Arechabala B, Coiffard C, Rivalland P, Coiffard LJ, De Roeck-Holtzhauer Y. **Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release.** J Appl Toxicol 1999;19(3):163-5.
We used the neutral red test, MTT assay and lactate dehydrogenase (LDH) release to compare the potential cytotoxicity of six surfactants belonging to different classes--three non-ionic surfactants (Triton x100, octylphenoxypolyethoxy alcohol, from Orion; Tween 60, polyoxyethylene (20) sorbitan monostearate, from ICI Speciality Chemicals; Tween 80, polyoxyethylene (20) sorbitan monolaurate, from Labosi), two anionic surfactants (Texapon K1298, sodium lauryl sulphate, from Henkel; Texapon N40, sodium laurylether sulphate, from Henkel) and one cationic surfactant (benzethonium chloride, from Siber Hegner)--on human fibroblast cultures. According to the LC50 (microg ml(-1)), the tested surfactants can be classified in the following order of increasing cytotoxicity: Tween 80 < Texapon N40 < Tween 60 < Texapon K1298 < Triton x100 < benzethonium chloride.

Benoit-Vical F, Valentin A, Mallie M, Bastide JM, Bessiere JM. **In vitro antimalarial activity and cytotoxicity of cochlospermum tinctorium and C. planchonii leaf extracts and essential oils [letter]**. Planta Med 1999;65(4):378-81.

The antimalarial and toxicological properties of Cochlospermum tinctorium and C. planchonii extracts and essential oils prepared from their leaves were studied. The oil components were extracted by hydrodistillation of the plant leaves and characterized by gas chromatography and mass spectrometry. Crude extracts and oils were tested for in vitro antimalarial activity on Plasmodium falciparum. The IC50 were evaluated after 24 and 72 h contact between the oils and the parasite culture, and ranged from 22 to 500 micrograms/ml. C. planchonii leaf oil yielded the best antimalarial effect (IC50: 22-35 micrograms/ml), while the most potent effect from crude leaf extracts was induced by C. tinctorium. The cytotoxicity of the leaf crude extracts and oils was assessed on the K562 cell line and showed IC50 values ranging between 33 and 2000 micrograms/ml.

Berger D, Citarella R, Dutia M, Greenberger L, Hallett W, Paul R, Powell D. **Novel multidrug resistance reversal agents.** J Med Chem 1999;42(12):2145-61.
A series of 59 alpha-aryl-alpha-thioether-alkyl, -alkanenitrile, and -alkanecarboxylic acid methyl ester tetrahydroisoquinoline and isoindoline derivatives (15a-48) were synthesized and evaluated as multidrug resistance (MDR) reversal agents. The compounds were tested on S1-B1-20 human colon carcinoma cells selected for resistance to bisantrene. Both the cytotoxicity of the reversal agents and their ability to resensitize the cells to bisantrene were determined. All but two of these compounds (15q, 40) were more effective MDR reversal agents in vitro than verapamil (VRP), a calcium channel antagonist which also has been shown to possess MDR modulating activity. Several showed good activity in this assay (IC50's < 0.5 microM), the most potent being isoindolines 44 (IC50 0.26 microM) and 46 (IC50 0.26 microM) and tetrahydroisoquinolines 47 (IC50 0.29 microM) and 15m (IC50 0.30 microM). A number of compounds were evaluated in vivo against vincristine (VCR)-resistant murine P388 leukemia, as well as against human epidermoid carcinoma KB/8.5 implanted sc in athymic mice. The reversal agents which consistently showed the highest activity, together with low toxicity, were alpha-aryl-alpha-thiotolylalkanenitrile tetrahydroisoquinoline derivatives with electron-rich alkoxy substituents on the aromatic rings. Of the tested compounds, the most effective reversal agents for both tumor lines were 15h (33% increased life span at 12.5 mg/kg, 0.2 mg/kg VCR versus VCR alone in the VCR-resistant P388 leukemia model and 59% relative tumor growth at 50 mg/kg, 8 mg/kg doxorubicin versus doxorubicin alone in the KB/8.5 model) and 39a (48% increased life span at 50 mg/kg, 0.2 mg/kg VCR versus VCR alone in the VCR-resistant P388 leukemia model and 46% relative tumor growth at 25 mg/kg, 8 mg/kg doxorubicin versus doxorubicin alone in the KB/8.5 model). The mechanism of action of these compounds is believed to involve blocking the drug efflux pump, P-glycoprotein.

Bremer S, Van Dooren M, Paparella M, Kossolov E, Fleischmann BK, Hescheler J. **Effects of embryotoxic chemicals on the in vitro differentiation of genetically engineered embryonic stem cells into cardiac cells.** Toxicol In Vitro 1999;13(4-5):645-50.
BIOSIS COPYRIGHT: BIOL ABS. A project has been started using transgenic embryonic stem cells as a toxicological endpoint in order to register chemical effects on the development of embryonic tissues which are known to be sensitive during their differentiation. The green fluorescent protein (GFP) is used as a reporter gene and is linked to a cardiac specific promotor. This construct is integrated into the native DNA of undifferentiated embryonic stem cells. The expression of GFP was switched on after specific activation of d to the IC50 values given by other in vitro endpoints in order to investigate the potential ofthis toxicological endpoint. The results show a higher sensitivity of endpoints which analysed specific

effects on a selected target tissue. The exposure of embryonic stem cells to chemicals lead to the following IC50 values: 1.149 : 0.170 ng/ml (cytotoxicity) versus 0.216 : 0.126 ng/ml (GFP expression) after treatment with retinoic acid and 54.2 : 5.2 ng/ml (cytotoxicity) versus 26.7 : 2 ng/ml (GFP ex.

Catala M, Anton A, Portoles MT. **Characterization of the simultaneous binding of Escherichia coli endotoxin to Kupffer and endothelial liver cells by flow cytometry.** Cytometry 1999;36(2):123-30.
BIOSIS COPYRIGHT: BIOL ABS. Background: The triggering of cellular responses during endotoxic shock is initiated for the binding of endotoxin (lipopolysaccharide; LPS) to the cell surface. Kupffer and endothelial liver cells, involved in the removal of endotoxin from blood circulation, show in vitro a rapid response to LPS in the absence of serum. Methods: A double-labeling fluorescent assay was designed to evaluate the binding properties of Escherichia coli 0111:B4 LPS to individual endothelial and Kupffer cells in suspen e binding of endotoxin was observed with both populations, showing properties of a receptor-mediated process. The Kupffer cell population showed a faster capacity and a higher affinity for LPS binding. The Hill coefficients indicated positive cooperativity in the LPS interaction with both populations. Conclusions: Specific endotoxin binding to liver sinusoidal cells occurs in a serum-independent manner, particularly at high LPS concentrations. Flow cytometry is a fast, precise, and efficient tec.

Clothier R, Starzec G, Stipho S, Kwong YC. **Assessment of initial damage and recovery following exposure of MDCK cells to an irritant.** Toxicol In Vitro 1999;13(4-5):713-7.
BIOSIS COPYRIGHT: BIOL ABS. The ability of Madin-Darby canine kidney cells (MDCK) to form cell tight-junctions and orientate correctly on porous membranes has been exploited to model corneal barrier function. While the ability to monitor recovery profiles has not yet been included in a prevalidation trial, its inclusion facilitates the prediction of potential adverse reactions. Combining a viability assay (Alamar blue reduction) with the fluorescein leakage assay, the chemical effects on cell membrane and adhesion molecule escein leakage gave recovery in 48 hours, 50% gave marginal recovery in 96 hours. Comparable effectswere noted for the restoration of the ability to reduce the Alamar blue dye. Resolution of the damaging effects of benzalkonium chloride and isopropanol took longer than for Tween 20, as is the case in the rabbit in vivo.

Coppola D, Saunders B, Fu L, Mao W, Nicosia SV. **The insulin-like growth factor 1 receptor induces transformation and tumorigenicity of ovarian mesothelial cells and down-regulates their Fas-receptor expression.** Cancer Res 1999;59(13):3264-70.
Cell proliferation and papillogenesis are growth factor-sensitive events in the ovarian mesothelium, the tissue source of ovarian epithelial cancer. To further investigate the regulation of cell proliferation in this tissue, rabbit ovarian mesothelial cells (OMC) were transfected in vitro with a CVN expression vector carrying the human gene for insulin-like growth factor 1 receptor (IGF-1R). The growth characteristics of IGF-1R transfectants (OMIR) and their response to IGF-1 were then compared with those of OMC in serumless HL-1 cultures. OMIR cells formed epithelial-like colonies and, even when nonconfluent, produced tridimensional structures reminiscent of papillae seen in ovarian serous epithelial tumors. After 3 and 7 days of exposure to IGF-1, OMIR cells grew approximately 20-fold ($P < 0.05$), and papillogenesis was 15- to 25-fold over similar events in OMC, respectively. Exposure to treatment with antisense oligonucleotides against IGF-1R mRNA inhibited OMIR growth rate by 70%. Western immunoblotting and flow cytometry revealed higher expression of IGF-1R in OMIR cells than in OMC.

The reverse was true when Fas-receptor expression was evaluated. OMIR cells were clonogenic in 15% serum-rich soft agar assay (OMIR:OMC colony-forming ratio 150-200:1), and tumorigenic in nude mice in which high-grade carcinomas with occasional lung metastases were observed. These data suggest that IGF-1R plays a role in ovarian epithelial carcinogenesis. The overexpression of this receptor induces transformation and morphogenesis of OMCs via an autocrine mechanism. IGF-1R may down-regulate the Fas expression rendering transformed ovarian mesothelial cells resistant to apoptosis.

Dahle J, Steen HB, Moan J. **The mode of cell death induced by photodynamic treatment depends on cell density.** Photochem Photobiol 1999;70(3):363-7.
Madison Darby canine kidney II (MDCK II) cells were seeded out at two different densities and incubated with 125 micrograms/mL of the photosensitizer meso-tetra(4-sulfonatophenyl)porphine (TPPS4) for 18 h, washed and irradiated with blue light. Four hours later the cells were studied by fluorescence microscopy. Apoptotic cells were detected by virtue of the distinct condensation and fragmentation of chromatin, and necrotic cells were detected by uptake of propidium iodide. In addition apoptosis was measured by the TdT assay. The fraction of apoptotic cells and the fraction of necrotic cells were determined for both cell densities at various levels of survival. With < 55% total cell death the apoptotic fraction was significantly higher for cells in confluent monolayers than for cells growing in microcolonies at equitoxic doses. Confluent cells were 2.9 times more sensitive than cells in microcolonies partly due to a 1.5 times higher uptake of TPPS4 in monolayer cells. The difference in mode of cell death for the different cell densities was not related to any observable difference in subcellular localization pattern of TPPS4 at equitoxic doses of photodynamic treatment.

Dartsch PC, Hildenbrand S, Gfroerer W, Kimmel R, Schmahl FW. **Cytotoxic effects of 2-butoxyethanol in vitro are related to butoxyacetaldehyde, an intermediate oxidation product.** Environ Toxicol Pharmacol 1999;7(2):135-42.
BIOSIS COPYRIGHT: BIOL ABS. Ethylene glycol ethers belong to a group of solvents with a wide spectrum of applications, particularly because of their compatibility to both hydrophilic and lipophilic systems. Especially ethylene glycol monobutyl ether (2-butoxyethanol, BE) is widely used as a key ingredient in many industrial and consumer cleaning products. Therefore, the risk of human exposure and toxicity by BE as well as its potential for environmental contamination have to be carefully evaluated. By using an established e the latter BE contained besides butyraldehyde and n-butanol 0.5 vol % butoxyacetaldehyde (BAL) asmeasured by capillary gas chromatography and mass spectrometry. Freshly used BE did not cause a toxic effect in the in vitro assays at all concentrations tested (up to 1 mg/ml). In contrast, stored BE which contained BAL reduced cell viability and mitotic activity in a dose-dependent manner. The effective concentration of stored BE causing a 50% loss in cell viability (EC50/24h) was calculated to be h of 15 mug/ml, which is a 70-fold lower concentration when compared with stored BE. The present study provides evidence that BE possesses only a low cytotoxic potential in vitro, whereas the corresponding BAL, an intermediate in the oxidation process of BE to butoxyacetic acid, has marked toxic effects. The occurrence of the aldehyde might explain the predominant hematological effects of BE observed in vivo.

Denecke J, Becker K, Jurgens H, Gross R, Wolff JE. **Falsification of tetrazolium dye (MTT) based cytotoxicity assay results due to mycoplasma contamination of cell cultures.** Anticancer Res 1999;19 (2a):1245-8₆

Mycoplasma contamination of cell cultures is a frequently observed problem. Due to the inconspicuous growth in cell cultures, periodical screening procedures represent the only protection. Many influences of mycoplasma on cell culture parameters have been described. We addressed the question of whether mycoplasma contamination affects the most frequently used cytotoxicity assay, the tetrazolium based MTT assay. We contaminated C6 glioma cells with mycoplasma and performed MTT assays with doxorubicin, vincristine, etoposide and cisplatinum under various conditions. Contaminated cells demonstrated significant different results when tested with the MTT assay than mycoplasma free controls. Differences were not detectable when cells were counted as toxicity assay. Due to an additional reduction of tetrazolium by mycoplasmas, contaminated cells appeared up to 15 fold resistant to doxorubicin, vincristine and etoposide, but not to cisplatinum. Differences decreased with decreasing drug doses and decreasing plated cell count. Our findings confirm the compelling need for periodical mycoplasma screening, especially when tetrazolium based cytotoxicity assay (MTT) are used.

Dias N, Nicolau A, Carvalho GS, Mota M, Lima N. **Miniaturization and application of the MTT assay to evaluate metabolic activity of protozoa in the presence of toxicants.** J Basic Microbiol 1999;39(2):103-8.
This paper describes a critical evaluation of a miniaturised colorimetric assay, using MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) reduction, applied to protozoan viability testing. The toxic substances used were copper, zinc, Triton X-100 (a membrane surfactant) and cycloheximide (an inhibitor of the protein synthesis). The viability assay of the ciliate protozoan Tetrahymena pyriformis was optimised in terms of MTT concentration and incubation time. Since protozoa are non adherent cells the MTT assay was modified in order to maintain the medium in the well. MTT proved to be effective in the measurement of Tetrahymena pyriformis viability. Four hours of MTT incubation followed by 30 minutes of incubation with DMSO were found to be the best incubation times for optical density reading. Furthermore, 10 mg/ml of MTT solution was the concentration that gave higher values of optical densities with minor medium interference.

Finlay WJ, Logan NA, Sutherland AD. **Semiautomated metabolic staining assay for Bacillus cereus emetic toxin.** Appl Environ Microbiol 1999;65(4):1811-2.
BIOSIS COPYRIGHT: BIOL ABS. This paper describes a specific, sensitive, semiautomated, and quantitative Hep-2 cell culture-based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for Bacillus cereus emetic toxin. Of nine Bacillus, Brevibacillus, and Paenibacillus species assessed for emetic toxin production, only B. cereus was cytotoxic.

Geldof AA, Mastbergen SC, Henrar RE, Faircloth GT. **Cytotoxicity and neurocytotoxicity of new marine anticancer agents evaluated using in vitro assays.** Cancer Chemother Pharmacol 1999;44 (4):312-8.
PURPOSE: New classes of anticancer drugs, isolated from marine organisms, have been shown to possess cytotoxic activity against multiple tumor types. Aplidine, didemnin B, and isohomohalichondrin B (IHB), among the more promising antitumor candidates, have been evaluated in the present study on a comparative basis in terms of their antiproliferative activity and neurotoxic effects in vitro. METHODS: Using a panel of different human, prostatic cancer cell lines (DU 145, PC-3 and LNCaP-FGC) the effects of Aplidine, didemnin B, and IHB on tumor cell proliferation were tested in a colorimetric (XTT) assay and compared with the effects of vincristine, vinorelbine, and Taxol. Under analogous in vitro

conditions these drugs were also monitored for neurocytotoxic effects using a PC 12 cell line based model. RESULTS: Didemnin B and - especially - Aplidine were more effective in the inhibition of prostate cancer cell proliferation than vincristine, vinorelbine or Taxol at concentration levels between 5 and 50 pmol/ml. At these same concentrations, however, Didemnin B and Aplidine were also most potent in the in vitro neurotoxicity assays. IHB was found to exert even more potent antiproliferative activity (at concentration levels between 0.05 and 0.1 pmol/ml). However, neurotoxic effects were also found to be present at these levels. After drug withdrawal, the neurotoxic damage, inflicted by aplidine or IHB appeared to be more long lasting than after vincristine or vinorelbine exposure. CONCLUSIONS: These results point to high antiproliferative activity of aplidine and IHB in prostate cancer. At the same time, the data urge some caution in the clinical use of these agents because of potential neurotoxic side-effects. The use of a newly formulated Aplidine may involve a more favorable therapeutic profile.

Hanley AB, McBride J, Oehlschlager S, Opara E. **Use of a flow cell bioreactor as a chronic toxicity model system.** Toxicol In Vitro 1999;13(4-5):847-51.
BIOSIS COPYRIGHT: BIOL ABS. We describe the use of a model system to mimic chronic toxin exposure, similar to that which might be found in a human situation, where exposure to dietary or environmental toxins occurs at a low level for an extended period of time. This is in contrast to the acute, immediately toxic dose effect usually observed in flask tissue culture. The apparatus used was a flow cell bioreactor in which cells can be cultured for lengthy periods of time as a continuous viable population. The compound used as ow that of the NOEL for periods of up to 4 weeks and the viability of the population determined using MTT, trypan blue and ATP assays.

Ilc K, Ferrero JM, Fischel JL, Formento P, Bryce R, Etienne MC, Milano G. **Cytotoxic effects of two gamma linoleic salts (lithium gammalinolenate or meglumine gammalinolenate) alone or associated with a nitrosourea: an experimental study on human glioblastoma cell lines.** Anticancer Drugs 1999;10(4):413-7.
Gamma linoleic acid (GLA) salts may exert a direct antiproliferative activity on tumor cells. The cytotoxicity is linked to the generation of conjugated dienes, peroxyl radicals and superoxide radicals. Lithium gammalinolenate (LiGLA) and meglumine gammalinolenate (MeGLA) have been recently developed for enhancing the water solubility of these compounds. MeGLA or LiGLA (10(-5) to 10(-4) mol/l) and fotemustine (Fote) (2 x 10(-6) to 2 x 10(-4) mol/l) were applied, alone or in combination, for up to 9 days to two human glioblastoma cell lines A172 and U373MG. Fote was applied first followed by LiGLA and/or MeGLA. Cytotoxicity was evaluated by the MTT test, and the effects of drug combinations were analyzed by the isobolographic representation according to the Chou and Talalay method (combination indexes). For both GLA salts, cytotoxicity was manifested after 4 days of cell exposure and with very sharp dose-response curves. Comparison of IC50 values indicated that MeGLA was more active than LiGLA. There was a constant reduction in IC50 values following an increase in exposure time for A172 cells: between 4 and 9 days of cell exposure, IC50 changed from 73 to 46 microM for LiGLA and from 49 to 31 microM for MeGLA ($p<0.05$). With U373MG cells, there was no influence of exposure duration on IC50 values. Combination index values indicated that association between Fote and GLA salts globally resulted in slightly antagonistic effects. These results may be useful for further development of GLA salts at the clinical level.

Kapahi P, Boulton ME, Kirkwood TB. **Positive correlation between mammalian life span and cellular resistance to stress.** Free Radic Biol Med 1999;26(5-6):495-500.
Identifying the mechanisms determining species-specific life spans is a central challenge in understanding the biology of aging. Cellular stresses produce damage, that may accumulate and cause aging. Evolution theory predicts that long-lived species secure their longevity through investment in a more durable soma, including enhanced cellular resistance to stress. To investigate whether cells from long-lived species have better mechanisms to cope with oxidative and non-oxidative stress, we compared cellular resistance of primary skin fibroblasts from eight mammalian species with a range of life spans. Cell survival was measured by the thymidine incorporation assay following stresses induced by paraquat, hydrogen peroxide, tert-butyl hydroperoxide, sodium arsenite and alkaline pH (sodium hydroxide). Significant positive correlations between cell LD90 and maximum life span were found for all these stresses. Similar results were obtained when cell survival was measured by the MTT assay, and when lymphocytes from different species were compared. Cellular resistance to a variety of oxidative and non-oxidative stresses was positively correlated with mammalian longevity. Our results support the concept that the gene network regulating the cellular response to stress is functionally important in aging and longevity.

Maravelias C, Dona A, Athanaselis S, Koutsogeorgopoulou L, Koutselinis A. **Immunomodulative effects of some drugs of abuse on human peripheral blood lymphocytes.** Vet Hum Toxicol 1999;41 (4):205-10.
The cytotoxic effects of opiates, cocaine and their metabolites on peripheral blood mononuclear cells from healthy volunteers, concerning cell viability, were studied in a wide range of concentrations (ranging from 10-(2) to 10-(8) M), by 2 colorimetric in vitro assays, the neutral red uptake assay and thiazolyl blue tetrazolium bromide assay. All tested drugs of abuse and their metabolites were non-cytotoxic at concentrations lower than 10-(5) M. The possible immunomodulative effects of these substances were evaluated through phytohemagglutinin-induced lymphocyte proliferation ([3H]-thymidine DNA incorporation assay) as well as by a 51Cr release natural killer assay. The results showed immunomodulative effects of all the opiates tested. Cocaine, freebase cocaine and benzoylecgonine produced a statistically non-significant decrease of phytohemagglutinin proliferation. Cocaine induced a statistically non-significant increase, whereas freebase cocaine and benzoylecgonine showed a non-significant decrease of natural killer cell activity.

Mirto H, Barrouillet MP, Henge-Napoli MH, Ansoborlo E, Fournier M, Cambar J. **Influence of uranium(VI) speciation for the evaluation of in vitro uranium cytotoxicity on LLC-PK1 cells.** Hum Exp Toxicol 1999;18(3):180-7.
Very few data are available concerning the in vitro toxicity of uranium. In this work, we have determined the experimental chemical conditions permitting the observation of uranium(VI) cytotoxicity on LLC-PK1 cells. Uranium solutions made either by dissolving uranyl acetate or nitrate crystals, or by complexing uranium with bicarbonate, phosphate or citrate ligands, were prepared and tested. Experiments demonstrated that only uranium solutions containing citrate and bicarbonate ligands concentrations tenfold higher than the metal, were soluble in the cell culture medium. Cytotoxicity studies of all these uranium compounds were performed on LLC-PK1 cells and compared using LDH release, neutral red uptake and MTT assays. Dose dependent cytotoxicity curves were only obtained

with uranium-bicarbonate medium. This study has revealed a toxicity of uranium-bicarbonate complexes for 24 h expositions and for concentrations ranging from 7 x 10(-4)-10(-3) M, under these conditions, the CI50 (cytotoxicity index) was evaluated between 8.5 and 9 x 10(-4) M. In contrast, we noticed a lack of cytotoxicity response for uranium(VI)-citrate complexes. Electron transmission microscopy studies revealed, when LLC-PK1 cells were exposed to the uranium-bicarbonate system, that uranium penetrated and precipitated within the cytoplasmic compartment. Morphological studies conducted with citrate complexes did not show any cellular intake of uranium.

Muller RH, Olbrich C. **Solid lipid nanoparticles: phagocytic uptake, in vitro cytotoxicity and in vitro biodegradation. Part 2**. Pharm Ind 1999;61(6):564-9.
IPA COPYRIGHT: ASHP Toxicity data for solid lipid nanoparticles compared to other colloidal carrier systems are discussed, a special assay for solid lipid nanoparticle degradation is described, and in vitro cytotoxicity data are compared with in vivo toxicity studies.

Nelson SK, Wataha JC, Lockwood PE. **Accelerated toxicity testing of casting alloys and reduction of intraoral release of elements.** J Prosthet Dent 1999;81(6):715-20.
STATEMENT OF PROBLEM: Short-term (72-168 hours) in vitro testing of dental casting alloys for cytotoxicity may not reflect in vivo biocompatibility. An accelerated test for evaluation of dental casting alloy cytotoxicity could help screen newly developed alloys more rapidly and accurately. PURPOSE: This study evaluated a method of accelerating alloy cytotoxicity by short-term conditioning of alloys. Cytotoxicity and mass release of these conditioned alloys were compared with alloys conditioned for 10 months or unconditioned alloys. The hypothesis was that a short-term conditioning procedure could be developed that would give cytotoxicity and mass release values similar to alloys exposed to a biologic solution for 10 months. MATERIAL AND METHODS: Dental casting alloys were conditioned in either saline, cell-culture medium, or a saline/bovine serum albumin (BSA) solution for 168 hours before standard in vitro cytotoxicity testing. Eight types of casting alloys with a range of nobilities (98% to 0%) were tested (n = 6). Controls were Teflon (Tf). Conditioned alloys were placed in direct contact with Balb/c fibroblasts for 72 hours, and cell viability was measured by succinic dehydrogenase activity (MTT method) relative to Tf controls. Elements released into the conditioning solutions were measured by atomic absorption spectroscopy. The cytotoxicities of conditioned alloys and total mass released were compared with unconditioned alloys (0 month) and alloys that were exposed to cell culture medium for 10 months. ANOVA and Tukey multiple comparison intervals (alpha =. 05) were used to compare mass released and cytotoxicity. RESULTS: Conditioning for 168 hours altered the cytotoxicity of the alloys. The saline/BSA conditioning solution reduced cytotoxicity of the alloys compared with unconditioned alloys, except for the Ni-Cr alloy. Other conditioning solutions were not as uniform in their effects, some increasing toxicity, others decreasing it. Overall, the saline/BSA solution was the most effective at changing alloy cytotoxicity from the unconditioned (0 month) toward the 10-month values. Mass loss during saline/BSA conditioning most closely approximated 10-month loss for most alloys. CONCLUSION: Conditioning of casting alloys appeared to be a useful method for predicting long-term cytotoxicity with a short-term in vitro test, but all conditioning solutions were not equivalent.

Nelson SK, Wataha JC, Neme AM, Cibirka RM, Lockwood PE. **Cytotoxicity of dental casting alloys pretreated with biologic solutions.** J Prosthet Dent 1999;81(5):591-6.
STATEMENT OF PROBLEM: Short-term (72-168 hours) in vitro tests are used to evaluate the

cytotoxicity of dental casting alloys. The ability of these short-term tests to predict long-term in vivo cytotoxicity has been questioned. A procedure to accelerate the testing of casting alloys would be useful in predicting longer-term alloy cytotoxicity. PURPOSE: This study hypothesized that preconditioning casting alloys by soaking in a biologic liquid would change subsequent cytotoxicity by removing some elements. Preconditioning may be 1 method of accelerating short-term in vitro tests. MATERIAL AND METHODS: Dental casting alloys were exposed to either saline, cell culture medium, or a saline/bovine serum albumin (BSA) solution for 72 hours before standard in vitro cytotoxicity testing. Six types of alloys were tested (n = 6): 5 Au-Ag-Cu-Pd alloys (single phase) and 1 Ag-Pd-Cu alloy (multiple phase). Teflon (Tf) samples served as a control. After preconditioning, alloys were placed in direct contact with Balb/c fibroblasts for 72 hours, after which cell viability was measured by succinic dehydrogenase activity (MTT method) relative to Tf controls (100% = no toxicity). Elements released into the preconditioning solutions were measured by atomic absorption spectroscopy. Cytotoxicities of preconditioned alloys and amounts of elemental release were compared with unconditioned alloys. RESULTS: A preconditioning time of 72 hours was sufficient to change the cytotoxicity of the tested alloys. The alloys that were more cytotoxic initially became less cytotoxic after preconditioning. For all the alloys tested, except the Ag-Pd-Cu multiphase alloy, preconditioning with either the saline or the saline/BSA solution caused an increase in cellular activity, therefore the preconditioned alloys were less cytotoxic. The cell culture medium preconditioning solution had a variable effect, causing increased or decreased cellular activity depending on the alloy treated. CONCLUSION: Preconditioning of casting alloys decreased subsequent cytotoxicity. However, not all preconditioning solutions are equivalent. A preconditioning strategy may be useful in accelerating the short-term cytotoxicity test toward a longer-term result.

Oakes DJ, Pollack JK. **Effects of a herbicide formulation, Tordon 75D, and its individual components on the oxidative functions of mitochondria.** Toxicology 1999;136(1):41-52.
This investigation evaluates the toxicity of a herbicide formulation, as well as testing its active and other components (other components comprise all components of Tordon 75D excluding the active components: i.e. the solvents, triisopropanolamine and diethyleneglycol monoethyl ether, a silicone defoamer and a proprietary surfactant, polyglycol 26-2). The results showed that Tordon 75D (a mixture of the triisopropanolamine salts of 2,4-dichlorophenoxy acetic acid (2,4-D) and 4-amino-3,5,6-trichloropicolinic acid (picloram) and its other components) impaired the oxidative functions of submitochondrial particles (SMPs). The effective concentrations that caused 50% inhibition of SMP activity (EC50s) for Tordon 75D were in the low micromolar range for 2,4-D and picloram in the presence of the other components, while in the absence of the other components exposure to 136 times higher concentrations of the triisopropanolamine forms of 2,4-D and picloram administered as a mixture were required to inhibit the oxidative functions of SMPs. Tordon 75D also significantly decreased the respiratory control ratio of intact rat liver mitochondria. The results show that the toxic effects of Tordon 75D on SMPs (at the EC50) and intact rat liver mitochondria were not due to any additive or synergistic actions of a mixture of its active and other components, but rather were caused solely by the proprietary surfactant. Since mitochondria are responsible for over 90% of the energy production in all eukaryotic organisms, the use of the SMP assay provides a convenient in vitro assay for evaluating cellular toxicity and can be regarded as an informative screening assay when designing chemical products which contain mixtures of chemicals.

Pioletti DP, Takei H, Kwon SY, Wood D, Sung KL. **The cytotoxic effect of titanium particles phagocytosed by osteoblasts.** J Biomed Mater Res 1999;46(3):399-407.
The cytotoxic effect of different concentrations of titanium particles on osteoblasts was studied in vitro. It was found that the viability of the osteoblasts was inversely proportional to the particle concentration. Phagocytosis of particles by the osteoblasts was evident and was demonstrated to be responsible for cell necrosis. Moreover, during and after phagocytosis, the osteoblasts released products that were cytotoxic for other osteoblasts, as established with a conditioned medium assay. The titanium particles thus had both a direct and an indirect effect on osteoblast viability. It also was observed that the titanium particles induced a process of programmed cell death (apoptosis) when co-cultured with osteoblasts. The results of this study suggest that not only is the amount of wear debris generated important, but the local accumulation of the debris also may have a significant impact on bone cell function. Copyright 1999 John Wiley & Sons, Inc.

Rasmussen E. **Use of fluorescent redox indicators to evaluate cell proliferation and viability.** In Vitro Mol Toxicol 1999;12(1):47-58.
BIOSIS COPYRIGHT: BIOL ABS. The performance of two cell viability test kits based on the use of redox indicators yielding fluorescent products, the AlamarBlue assay and a resazurin-based in vitro toxicology assay kit from Sigma, was compared in the present study. Cultures of human neonatal foreskin fibroblasts were exposed to equal concentrations of the two dye solutions in the cell culture media. The fluorescence intensities of the cell culture media obtained in response to cell proliferation with the two dyes showed a pr components were tentatively identified as resazurin and resorufin. The AlamarBlue assay has gainedwide application as a cell viability indicator that allows continuous monitoring of cell proliferation or cytotoxicity in human and animal cells, bacteria, and fungi, but no studies with the deliberate use of resazurin reduction to measure cell proliferation in cultures of somatic mammalian cells have been published. In the AlamarBlue dye solution, resazurin is supplemented with various stabilizing.

Roden MM, Lee KH, Panelli MC, Marincola FM. **A novel cytolysis assay using fluorescent labeling and quantitative fluorescent scanning technology.** J Immunol Method 1999;226(1-2):29-41.
BIOSIS COPYRIGHT: BIOL ABS. A novel cellular cytotoxicity assay using Calcein acetoxymethyl (Calcein-AM), a cytoplasmic fluorescent label, has been developed as an alternative to the standard 51Chromium (Cr)-release. Target cells were loaded with Calcein-AM and then co-incubated with effector cells. An additional reagent, FluoroQuench, is added to extinguish fluorescence of dying target cells and of the culture media. Assay plates are read on a quantitative fluorescent scanner for determination of viable target cells. Perc Cr-release assay, the Calcein assay reliably measures cell-mediated cytotoxicity with little variance among replicates. The fluorescent assay represents a simple and useful alternative to the use of radioactive materials and adds the additional benefit of digital images and analysis.

Rodriguez JA, Haun M. **Cytotoxicity of trans-dehydrocrotonin from Croton cajucara on V79 cells and rat hepatocytes.** Planta Med 1999;65(6):522-6.
The cytotoxicity of trans-dehydrocrotonin (DHC), an antiulcerogenic diterpene from Croton cajucara (Euphorbiaceae), was assessed on a V79 fibroblast cell line and on rat hepatocytes. Three independent

endpoints for cytotoxicity were evaluated: DNA content, MTT reduction and neutral red uptake (NRU). For the V79 cells IC50 values of 253 and 360 microM were obtained for the NRU and MTT tests. The cytotoxic effect of DHC was time exposure dependent and no ability to recover after treatment was observed. For the rat hepatocytes IC50 values of 8, 300 and 400 microM for the MTT, DNA and NRU assays were obtained. The greater toxicity observed for the MTT test was inhibited when the experiment was performed using non-fresh hepatocytes in an age-dependent fashion. The treatment of V79 cells with the conditioned medium resulting after hepatocyte incubation with DHC showed an enhancement of MTT reduction without any evident toxic effects on fibroblasts. These results suggest that DHC has basal cytotoxic effects as observed on V79 fibroblasts and expresses a selective cytotoxicity after its metabolization by the hepatocytes. The bioactivation of DHC is mediated by cytochrome P450 and could generate metabolites that have no toxicity for V79 fibroblasts.

Rumbaut RE, Sial AJ. **Differential phototoxicity of fluorescent dye-labeled albumin conjugates.** Microcirculation 1999;6(3):205-13.
OBJECTIVE: Fluorescent dyes are commonly used as probes for assessment of macromolecular permeability. Despite numerous examples of light-dye induced toxicity in the microvasculature, little is known regarding the relative phototoxicity of commonly used fluorescent conjugates. We, therefore, compared the phototoxicity of four fluorescent conjugates of bovine serum albumin (BSA) available commercially. METHODS: An in vitro photohemolysis assay was used, in which rat erythrocytes were incubated with fluorescently labeled BSA and exposed to epi-illumination, using an inverted microscope designed for microvascular permeability experiments. Photohemolysis was quantified by monitoring light transmission across the cells. RESULTS: Photohemolysis was dependent on excitation light intensity and fluorochrome concentration. Fluorescein isothiocyanate (FITC)-labeled BSA was the most phototoxic compound tested, inducing 50% of maximum response in 14 min. The relative phototoxicity of the BSA conjugates was: FITC > BODIPY-FL > Texas Red > tetramethylrhodamine isothiocyanate. The phototoxicity of FITC-BSA was related to a high molar dye content. Photohemolysis with each of the conjugates was inhibited by histidine, a singlet oxygen quencher. CONCLUSIONS: Relative phototoxicity of fluorescent albumin conjugates differs considerably. Selection of fluorescent conjugates for use in microvascular experiments based on phototoxicity should consider both the type and molar content of the fluorochrome.

Sinclair GM, Paton GI, Meharg AA, Killham K. **Lux-biosensor assessment of pH effects on microbial sorption and toxicity of chlorophenols.** FEMS Microbiol Lett 1999;174(2):273-8.
Lux-marked bacterial biosensors and a commercial toxicity testing bacterial strain (Microtox) were exposed to 2,4-dichlorophenol (DCP) and the light output response measured. Increasing DCP concentrations caused a decrease in light output in all three biosensors with an order of sensitivity (in terms of luminescence decrease over the DCP concentration range) of Pseudomonas fluorescens < Escherichia coli < Microtox. Adsorption of DCP to E. coli was measured using uniformly ring labelled [14C]DCP and found to be very rapid. The effect of pH on toxicity and adsorption was also investigated. Low pH values increased the amount of DCP adsorbed to the cell and increased the toxicity of DCP.

Stammati A, Bonsi P, Zucco F, Moezelaar R, Alakomi HL, Von Wright A. **Toxicity of selected plant volatiles in microbial and mammalian short-term assays.** Food Chem Toxicol 1999;37(8):813-23.
In this study, several short-term microbial and mammalian in vitro assays were used to evaluate

cytotoxicity and genotoxicity of four plant volatiles showing antifungal activity: cinnamaldehyde, carvacrol, thymol and S(+)-carvone. All inhibited viability and proliferation of Hep-2 cells in a dose-dependent manner. IC50 ranged from 0.3 mM (cinnamaldehyde) to 0.7 mM (thymol) in viability tests and from 0.2 mM (carvacrol) to 0.9 mM (carvone) in the proliferation test. The morphological analysis suggested an involvement of apoptosis in the cases of carvone, carvacrol and cinnamaldehyde. At nontoxic doses, carvacrol and thymol increased the number of revertants in the Ames test by 1.5-1.7 times, regardless of metabolic activation. In the SOS-chromotest, none of the four plant volatiles caused DNA damage at non-toxic doses. In the DNA repair test, a marked dose-dependent differential toxicity was observed with carvone and, to a lesser extent, with cinnamaldehyde, while with thymol and carvacrol, this effect was less pronounced. In conclusion, the considered in vitro cytotoxicity assays have shown to be sensitive enough to highlight a variety of toxic effects at the cellular level, which can be rather different between chemically closely related compounds, such as isomers.

Sung HW, Huang RN, Huang LL, Tsai CC. **In vitro evaluation of cytotoxicity of a naturally occurring cross-linking reagent for biological tissue fixation.** J Biomater Sci Polym Ed 1999;10 (1):63-78.
A recognized drawback of the currently available chemical cross-linking reagents used to fix bioprostheses is the potential toxic effects a recipient may be exposed to from the fixed tissues and/or the residues. It is, therefore, desirable to provide a cross-linking reagent which is of low cytotoxicity and may form stable and biocompatible cross-linked products. To achieve this goal, a naturally occurring cross-linking reagent -- genipin -- which has been used in herbal medicine and in the fabrication of food dyes, was used by our group to fix biological tissues. The study was to assess the cytotoxicity of genipin in vitro using 3T3 fibroblasts (BALB/3T3 C1A31-1-1). Glutaraldehyde, the most commonly used cross-linking reagent for tissue fixation, was used as a control. The cytotoxicity of the glutaraldehyde- and genipin-fixed tissues and their residues was also evaluated and compared. The observation in the light microscopic examination revealed that the cytotoxicity of genipin was significantly lower than that of glutaraldehyde. Additionally, the results obtained in the MTT assay implied that genipin was about 10000 times less cytotoxic than glutaraldehyde. Moreover, the colony forming assay suggested that the proliferative capacity of cells after exposure to genipin was approximately 5000 times greater than that after exposure to glutaraldehyde. It was noted that the cells seeded on the surface of the glutaraldehyde-fixed tissue were not able to survive. In contrast, the surface of the genipin-fixed tissue was found to be filled with 3T3 fibroblasts. Additionally, neocollagen fibrils made by these fibroblasts were observed on the genipin-fixed tissue. This fact suggested that the cellular compatibility of the genipin-fixed tissue was superior to its glutaraldehyde-fixed counterpart. Also, the residues from the glutaraldehyde-fixed tissue markedly reduced the population of the cultured cells, while those released from the genipin-fixed tissue had no toxic effect on the seeded cells. In conclusion, as far as cytotoxicity is concerned, genipin is a promising cross-linking reagent for biological tissue fixation.

Tang AT, Liu Y, Bjorkman L, Ekstrand J. **In vitro cytotoxicity of orthodontic bonding resins on human oral fibroblasts.** Am J Orthod Dentofacial Orthop 1999;116(2):132-8.
Polymerization of bonding resins is compromised by atmospheric oxygen, giving rise to a layer of low molecular weight chemical species commonly known as the oxygen inhibited layer. The aim of this study was to evaluate the cytotoxic effect of this layer on primary cultures of human oral fibroblast. The

cytotoxic effect related to the modes of polymerization of seven commercially available orthodontic bonding resins was also evaluated statistically. Each material was polymerized into 12 resin disks of standardized dimensions. Half of them were washed with 99% acetone to remove the oxygen inhibited layer. In duplicates, human oral fibroblasts were exposed to the intact and washed resin disks in tissue culture inserts. Cell viability was assessed by tetrazolium bromide reduction assay (MTT) 1, 3, and 6 days after exposure. Glass disks served as controls. ANOVA was used to test for statistical significance. Overall, the presence of an oxygen inhibited layer renders bonding resins 33% more cytotoxic ($P < .01$, $F = 11.83$, $DF = 1$). Light-cured and chemically cured 2-pastes materials had their mean cytotoxicities approximating their inert controls over 6 days. In chemically cured liquid-paste materials, the viability of human oral fibroblasts was only 37% ($P < .001$, $F = 26.4$, $DF = 2$) comparing to the control, 64% on day 1, 30% on day 3 and 14% on day 6. This suggested that the oxygen inhibited layer formed on the surface of bonding resins is an important cytotoxic source in vitro. Chemically cured liquid-paste materials were more cytotoxic than light-cured and chemically cured 2-paste materials. Further investigation into the influence of the modes of polymerization on materials' toxicodynamic effect is warranted to verify its clinical implication.

Van Den Heuvel RL, Leppens H, Schoeters GE. **Lead and catechol hematotoxicity in vitro using human and murine hematopoietic progenitor cells.** Cell Biol Toxicol 1999;15(2):101-10.
In vitro cloning assays for hematopoietic myeloid and erythroid precursor cells have been used as screening systems to investigate the hematotoxic potential of environmental chemicals in humans and mice. Granulocyte-monocyte progenitors (CFU-GM) from human umbilical cord blood and from mouse bone marrow (Balb/c and B6C3F1) were cultured in the presence of lead and the benzene metabolite catechol. Erythroid precursors (BFU-E) from human umbilical cord blood were cultured in the presence of lead. The in vitro exposure of the human and murine cells resulted in a dose-dependent depression of the colony numbers. The concentration effect relationship was studied. Results showed that: (1) Based on calculated IC50 values, human progenitors are more sensitive to lead and catechol than are murine progenitors. The dose that caused a 50% decrease in colony formation after catechol exposure was 6 times higher for murine cells (IC50 = 24 micromol/L) than for human cord blood cells (IC50 = 4 micromol/L). Lead was 10-15 times more toxic to human hematopoietic cells (IC50 = 61 micromol/L) than to murine bone marrow cells from both mice strains tested (Balb/c, IC50 = 1060 micromol/L; B6C3F1, IC50 = 536 micromol/L). (2) A lineage specificity was observed after exposure to lead. Human erythroid progenitors (hBFU-E) (IC50 = 3.31 micromol/L) were found to be 20 times more sensitive to the inhibitory effect of lead than were myeloid precursors (hCFU-GM) (IC50 = 63.58 micromol/L). (3) Individual differences in the susceptibility to the harmful effect of lead were seen among cord blood samples. (4) Toxicity of lead to progenitor cells occurred at environmentally relevant concentrations.

Van Kooten TG, Klein CL, Wagner M, Kirkpatrick CJ. **Focal adhesions and assessment of cytotoxicity.** J Biomed Mater Res 1999;46(1):33-43.
Focal adhesions are highly ordered assemblies of transmembrane receptors, extracellular matrix proteins, and a large number of cytoplasmic proteins, including structural proteins, as well as tyrosine kinases, phosphatases, and their substrates. They are now accepted as a prime component of signal transduction. Because focal adhesions also play an important role in cell morphology and migration, it can be argued that their presence is indicative of healthy cells. This has been the reason for several

research groups to conclude that biomaterials sustaining focal adhesion assembly are biocompatible. In this study we demonstrate that cells under cytotoxic stress may still be able to retain their focal adhesions. Human umbilical vein endothelial cells at passage 2 were exposed to nickel and zinc ion solutions ranging from 1 to 0.01 mM for 4 and 24 h. Cells were seeded on fibronectin precoated glass slides or in tissue culture quality 96-well plates. MTT conversion with 1 and 0.5 mM nickel and zinc was strongly depressed, indicating that these concentrations are cytotoxic. Proliferative activity was also affected by these concentrations. Cells exposed to zinc typically retracted and detached from the surface, whereas cells exposed to nickel remained on the surface without signs of retraction. Nevertheless, cells exposed to nickel were impaired to reach confluency, which was determined by cadherin-5 expression. All these data indicate that nickel ions at a sufficient concentration influence cells in a cytotoxic way. Despite this apparent cytotoxicity, focal adhesion distribution as visualized by immunofluorescence staining of vinculin was not affected. With zinc the morphological changes were accompanied by apparent fusion of focal adhesions during retraction and finally dissolution. These data indicate that the mere presence of focal adhesions does not allow a reliable statement about the functional status of a cell. On the other hand, when focal adhesions are affected it is an excellent monitor of disturbed cell function.

Velarde G, Ait-Aissa S, Gillet C, Rogerieux F, Lambre C, Vindimian E, Porcher JM. **Use of the CaCo-2 model in the screening of polluting substance toxicity.** Toxicol In Vitro 1999;13(4-5):719-22.
BIOSIS COPYRIGHT: BIOL ABS. The aim of this work was to investigate the oral toxicity of representative chemicals chosen from each class of the list of 132 substances present in industrial effluents after the EEC Directive 76-464. Owing to its characterization as a model of the intestinal epithelium, the CaCo-2 cell line model was chosen. Cytotoxicity was assayed using the tetrazolium blue (MTT) test. For most of the substances, a linear correlation was observed between the octanol/water partition coefficient (log Kw) and account only the non-ionized, lipid insoluble, concentration at pH 7.3. The amines still did notfit into the correlation, reinforcing the idea of a non-narcotic mechanism. The toxicity of a large number of substances can thus be predicted from their physico-chemical properties only when the substances exert a direct and non-specific effect. The amines appeared more toxic than substances with the same partition coefficient, showing that knowledge of the only lipophilicity is too restrictive to pr.

Vogel EW, Graf U, Frei HJ, Nivard MM. **The results of assays in Drosophila as indicators of exposure to carcinogens.** IARC Sci Publ 1999;(146):427-70.
Drosophila has fulfilled a dual function in the field of genetic toxicology: for use in short-term tests for identifying carcinogens and in a model for studies of the mechanisms of mutagenesis by chemicals. Until the mid-1980s, use of Drosophila in short-term tests was restricted to assays for genetic damage in germ cells, mostly in males. The largest database, on 700-750 chemicals, is available for the test for sex-linked recessive lethal (SLRL) forward mutation. The database for assays of the consequences of chromosomal breakage--reciprocal translocations and chromosome loss--is smaller, with about 100 chemicals tested. Comparative studies conducted within the US National Toxicology Program showed that SLRL is a better end-point than reciprocal translocation: of 66 chemicals (68 entries) that induced SLRL, only 28 (41%) induced reciprocal translocation. The major weakness of the SLRL assay is its low sensitivity (0.27-0.79) for mammalian genotoxins. A strength of the SLRL mutation test is its high specificity, which is close to 1. Thus, whereas a negative response in Drosophila provides little evidence

for genotoxicity, a positive response (SLRL frequency > or = five times the control level) provides good evidence that a chemical is a trans-species mutagen and probably also carcinogenic to mammals. The poor performance of the SLRL test revealed in several collaborative studies led to the development of assays for recombination in somatic cells of Drosophila. Two of these tests have been evaluated for all known classes of genotoxic chemical: the mwh/flr wing spot test on more than 400 chemicals and the white/white+ eye spot test on about 220 chemicals. Of 24 carcinogens that gave negative or inconclusive test results in the SLRL assay, 22 gave positive results in one or both of the somatic systems. Their better performance in comparison with the germ-line assays is primarily the result of their low cost (5-10% of that needed for an SLRL assay), allowing use of multiple doses and protocols and the use of distinct tester strains with heterogeneity for activation of procarcinogens. For qualitative and quantitative studies on structure-activity and activity-activity relationship, only germ-line system have been used. In general, clear relationships between physico-chemical parameters (s values, O6/N7-alkylguanine ratios), carcinogenic potency in rodents and several descriptors of genotoxic activity in germ cells (from mice and Drosophila) became apparent when the following descriptors were used: (1) estimates of TD50 (lifetime doses expressed in milligrams per kilogram body weight or millimoles per kilogram body weight) from bioassays for cancer in rodents; (2) the degree of germ-cell specificity, i.e. the ability of a genotoxic agent to induce mutations at practically any stage of development of Drosophila and mouse spermatogenesis, as opposed to a more specific response in postmeiotic stages of both species; (3) the M (NER-)/M(NER+) hypermutability ratio, determined in a repair assay in Drosophila germ cells; (4) the ratio of chromosomal aberrations to SLRL in postmeiotic germ cells of Drosophila, i.e. the comparative efficiency of a carcinogen to induce these two end-points; (5) mutational spectra induced at single loci, i. e. the seven loci used in the specific-locus test in mice and the vermilion, white and rosy genes of Drosophila; and (6) the doubling doses in milligrams or millimoles per kilogram for specific locus induction in mice. On the basis of these parameters, alkylating agents were classified into three categories in terms of germ-cell specificity, which is primarily due to stage-related differences in DNA repair, clastogenic efficiency, type of mutation spectra and carcinogenic potency in rodents. The three categories allow predictions of the genotoxicity of alkylating agents but not yet for other categories of genotoxic carcinogens.

Xu X, Hakansson L. **Simultaneous analysis of eosinophil and neutrophil adhesion to plasma and tissue fibronectin, fibrinogen, and albumin.** J Immunol Methods 1999;226(1-2):93-104.
A simple and convenient assay for the simultaneous measurement of eosinophil and neutrophil adhesion is described. Incubations were performed in microtitre plates coated with different proteins. Adhesion of eosinophils and neutrophils was determined by the use of specific radioimmunoassays for eosinophil cationic protein (ECP) and myeloperoxidase (MPO). Using this assay, Mn2+ induced a significant increase of the adhesion of eosinophils to plasma fibronectin and fibrinogen in a time-dependent fashion, while a small increase of the adhesion of neutrophils to these two proteins was observed. In contrast, a time-dependent potent increment of the adhesion of both eosinophils and neutrophils to tissue fibronectin and albumin was found. Tissue fibronectin preferentially supported eosinophil adhesion compared with that of neutrophils in the presence of Mn2+. PMA (10(-9) mol/l) induced a significant increase in the adhesion of eosinophils and neutrophils of the same pattern to all four proteins. However, when granulocytes were stimulated by Mn2+ in combination with PMA, eosinophils and neutrophils showed different patterns of response to plasma fibronectin and fibrinogen, respectively, but the same

pattern of response to tissue fibronectin. f-MLP stimulated an early increase of the adhesion of neutrophils to fibrinogen, while a weak stimulation of the adhesion of eosinophils to plasma fibronectin and fibrinogen and of neutrophils to plasma fibronectin was observed. Co-stimulation with f-MLP and Mn2+ did not induce any additive effects on granulocyte adhesion. In conclusion, the assay allows rapid quantification of eosinophil and neutrophil adhesion and can be used to directly compare the response of neutrophils and eosinophils. The assay is thus suitable for studies aimed at identifying agents with a selective effect on either of the cells.

Yang SW, Abdel-Kader M, Malone S, Werkhoven MC, Wisse JH, Bursuker I, Neddermann K, Fairchild C, Raventos-Suarez C, Menendez AT, et al. **Synthesis and biological evaluation of analogues of cryptolepine, an alkaloid isolated from the Suriname rainforest.** J Nat Prod 1999;62(7):976-83.
Bioassay-guided fractionation of an extract of a mixture of Microphilis guyanensis and Genipa americanacollected in the rainforest of Suriname yielded the known alkaloid cryptolepine (2) as the major active compound in a yeast bioassay for potential DNA-damaging agents; the same compound was later reisolated from M. guyanensis. The structure of cryptolepine was identified unambiguously by spectral data and by its total synthesis. Several cryptolepine derivatives (3-29, 32-41) were synthesized based on modifications of the C-2, N-5, N-10, and C-11 positions. Two cryptolepine dimers (30, 31) were also prepared. The structure modifications did not result in compounds with a higher potency than the parent compound cryptolepine in the yeast assay system, although some derivatives did show significant activity. Selected compounds (6, 7, 17, 22, 23, 26, and 27) were also tested for cytotoxicity in mammalian cell culture, and two compounds showed significant cytotoxic activity.

DERMAL TOXICITY

Basketter DA, Flyvholm MA, Menne T. **Classification criteria for skin-sensitizing chemicals: a commentary.** Contact Dermatitis 1999;40(4):175-82.
A formalized, standardized and effective mechanism for the identification of substances which possess significant skin sensitization potential is a necessary first step in the process of limiting the incidence of allergic contact dermatitis. Strategies to achieve such hazard identification are unified throughout the European Union and also have been publicised by the World Health Organization. Global harmonization of these and other approaches (e.g., in the USA) is being driven by the Organization for Economic Cooperation and Development. In this paper, the benefits and limitations of these classification systems are reviewed. A common element of all the systems is that they seek to distinguish important contact allergens from chemicals which are infrequent sensitizers. The European criteria are legal requirements in the EU member states and formal classification as a skin sensitizer leads to mandatory labelling. The most notable omission from current and proposed classification criteria relates to the relative potency of a classified skin sensitizer and the exposure dose. Such information is necessary for proper risk assessment and management measures to be implemented.

Bernhofer LP, Barkovic S, Appa Y, Martin KM. **IL-1alpha and IL-1ra secretion from epidermal equivalents and the prediction of the irritation potential of mild soap and surfactant-based consumer products.** Toxicol In Vitro 1999;13(2):231-9.
BIOSIS COPYRIGHT: BIOL ABS. We have previously evaluated the measurement of viability and

cytokine release from skin equivalents, for predicting the skin irritation potential of topically applied surfactants and demonstrated that IL-1alpha and interleukin-1 receptor antagonist (IL-1ra) release from epidermal skin equivalents correlates with skin irritation potential. In this study, the utility of the model was confirmed by the evaluation of cleansing bars and cleansing lotions that exhibited varying degrees of irritation p uman irritation data, demonstrating that the model can correctly predict the irritation potentialof soap and surfactant products. These results show that this in vitro model is useful for rank ordering the irritation potential of mild consumer products and for demonstrating enhanced mildness in products with minor differences.

Bernhofer LP, Seiberg M, Martin KM. **The influence of the response of skin equivalent systems to topically applied consumer products by epithelial-mesenchymal interactions.** Toxicol In Vitro 1999;13(2):219-29.
BIOSIS COPYRIGHT: BIOL ABS. A number of diverse in vitro model systems have been employed for the prediction of irritation potential of test articles. Monolayer systems have proven to be useful for preliminary screening but are not always capable of distinguishing mild effects or adaptable to fully formulated product. Three-dimensional reconstructed skin equivalents integrate cellular toxicity with the kinetics of exposure and absorption, serving as more realistic models; however, it is not obvious which of the three-dimen endent on keratinocyte-fibroblast interactions. The most predictive combinations of model systems and biomarkers for each product category were identified following comparison to preclinical data and human in vivo skin responses. Using a panel of representative consumer products, we identified IL-1alpha, IL-1ra, IL-8 and GM-CSF release from skin equivalents as being the best indicators of irritation.

Bhatia KS, Singh J. **Mechanism of transport enhancement of LHRH through porcine epidermis by terpenes and iontophoresis: permeability and lipid extraction studies**. Pharm Res 1998 Dec;15:1857-62.
IPA COPYRIGHT: ASHP The effects of 5% terpenes/ethyl alcohol (ethanol) and iontophoresis on the in vitro permeation of gonadorelin (LHRH; luteinizing hormone releasing hormone) through porcine epidermis were studied in vitro. Terpenes/ethyl alcohol increased gonadorelin permeation by enhancing the extraction of stratum corneum lipids. Iontophoresis synergistically enhanced drug permeation through terpenes/ethyl alcohol treated epidermis.

Chen T, Segall EM, Langer R, Weaver JC. **Skin electroporation: rapid measurements of the transdermal voltage and flux of four fluorescent molecules show a transition to large fluxes near 50 V**. J Pharm Sci 1998 Nov;87:1368-74.
IPA COPYRIGHT: ASHP To study skin electroporation, a flow-through sampling system was used to measure the response of human skin in vitro to a series of exponential pulses for 4 negatively charged, hydrophilic fluorescent tracer molecules (molecular weights of 450-625 Da). Although differences in their molecular transport profiles were observed, all 4 molecules exhibited a transition from small to large fluxes at peak dermal voltages of about 50 V. This behavior may have reflected a transition from electroporation of the skin's appendages to electroporation of the multilamellar bilayer membranes within the stratum corneum.

Damour O, Augustin C, Black AF. **Applications of reconstructed skin models in pharmaco-**

toxicological trials. Med Biol Eng Comput 1998;36(6):825-32.
The development of new cosmetic formulations requires precise assessment of their safety and efficacy. Today, legislation demands quality control combined with severe safety measures, as well as a limited use of animals for such testing (European Community directive 93/35/EEC). Consequently, safety assessment protocols are oriented towards in vivo tests on human volunteers and in vitro alternative methods to animal use, especially tissue engineered skin substitutes. In this paper, dermal and skin equivalents developed in the laboratory are described. The applications of reconstructed epidermis and skin substitutes for pharmaco-toxicological trials are also discussed. These tissue models have been shown to be very useful tools to assess cutaneous irritation, phototoxicity, photoprotection and to perform efficacy tests of cosmetic molecules and finished products. In conclusion, the authors are confident that these in vitro models can contribute to reduce animal use for routine toxicity testing.

De Brugerolle De Fraissinette A, Picarles V, Chibout S, Kolopp M, Medina J, Burtin P, Ebelin ME, Osborne S, Mayer FK, Spake A, et al. **Predictivity of an in vitro model for acute and chronic skin irritation (SkinEthic) applied to the testing of topical vehicles.** Cell Biol Toxicol 1999;15(2):121-35.
BIOSIS COPYRIGHT: BIOL ABS. An in vitro human reconstructed epidermis model (SkinEthic) used for screening acute and chronic skin irritation potential was validated against in vivo data from skin tolerability studies. The irritation potential of sodium lauryl sulfate (SLS), calcipotriol and trans-retinoic acid was investigated. The in vitro epidermis-like model consists of cultures of keratinocytes from human foreskin on a polycarbonate filter. The modulation of cell viability, the release and gene expression of proinflamm s. All topical products that were nonirritating in the human study were noncytotoxic and did not induce cytokine expression in the in vitro acute model (day 1 exposure). All irritating controls exhibited specific cell viability and cytokine patterns, which were predictive of the in vivo human data. The ranking of mild to moderate skin irritation potential was based on the lack of cytotoxicity and the presence of cytokine patterns including gene expression specific for each irritant, using the ch.

Fickweiler S, Abels C, Karrer S, Baumler W, Landthaler M, Hofstadter F, Szeimies RM. **Photosensitization of human skin cell lines by ATMPn (9-acetoxy-2,7,12,17-tetrakis-(beta-methoxyethyl)-porphycene) in vitro: mechanism of action.** J Photochem Photobiol B 1999;48(1):27-35.
9-Acetoxy-2,7,12,17-tetrakis-(beta-methoxyethyl)-porphycene (ATMPn) is a promising new photosensitizer characterized by high absorption around 640 nm and high singlet oxygen yield. To study the mechanism of action in vitro we have investigated uptake, intracellular localization, cell survival and ultrastructural changes following photodynamic treatment in human cell lines derived from the skin (SCL1 and SCL2, squamous cell carcinoma; HaCaT keratinocytes; N1 fibroblasts). Using flow cytometry we have determined the cellular fluorescence as a marker for the uptake of ATMPn after incubation for 60 min. Co-staining with ATMPn and fluorescent dyes specific for cell organelles reveals an intracellular localization of ATMPn in lysosomes. Following irradiation using an incoherent light source (580-740 nm) and a light fluence of 24 J cm-2, phototoxicity is determined by means of the 3-4.5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay. For all cell lines ATMPn concentrations above 15 nM yield a significant phototoxic effect. The 50% effective concentration, EC50, for SCL1 cells is 11.2 +/- 2.9 nM ATMPn. ATMPn uptake and phototoxicity are more effective for HaCaT and

SCL1 as compared to SCL2 and N1 cells. Growth curves confirmed the results of the MTT assay. Because of the high lysosomal accumulation of ATMPn, already low photosensitizer concentrations without dark toxicity yield a high photodynamic effect. Immunofluorescence and electron microscopy reveal damage to tonofilaments, plasma membrane and mitochondria, indicating a mechanism unrelated to apoptosis. A dose yielding complete cell killing, as needed for oncological indications, might lead to necrosis, whereas lower sub-lethal doses result in induction of apoptosis.

Haigh JM, Beyssac E, Chanet L, Aiache JM. **In vitro permeation of progesterone from a gel through the shed skin of three different snake species**. Int J Pharm 1998 Aug 15;170:151-6.
IPA COPYRIGHT: ASHP The in vitro diffusion of 4% progesterone (Crinone) from a commercially available gel formulation through the dorsal and ventral portions of shed skin of 3 snake species was studied over a 72 h period. Considerable differences were apparent between the dorsal and ventral sites and between the different species of snake. The dorsal area showed better permeability for progesterone and the permeability order for the different species was python>cobra>viper. These differences may have been due to the thickness of the skin and the hinge:scale ratio. The results indicated that shed snake skin is not a model membrane for human skin.

Herouet C, Cottin M, Galanaud P, Leclaire J, Rousset F. **Contact sensitizers decrease 33D1 expression on mature Langerhans cells.** Eur J Dermatol 1999;9(3):185-90.
Langerhans cells play a critical role in allergic contact hypersensitivity. In vivo, these cells capture xenobiotics that penetrate the skin and transport them through the lymphatic vessels into regional lymph nodes for presentation to T cells. During this migration step, Langerhans cells become mature dendritic cells according to their phenotype and their high immunostimulatory capacity. In vitro, when isolated from the skin and cultured for 3 days, Langerhans cells undergo similar phenotypic and functional maturation. In this study, the capacity of sensitizers, irritants and neutral chemicals to modulate the surface marker expression and morphology of pure mature murine Langerhans cells in vitro was examined. Contact with 4 sensitizers (2,4-dinitrobenzenesulfate, 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, p-phenylenediamine, mercaptobenzo-thiazole) resulted in a rapid, specific, marked fall in 33D1 expression, a murine specific dendritic cell marker. No effect was observed with 2 neutral chemicals (sodium chloride, methyl nicotinate) or 2 irritants (dimethyl sulfoxide, benzalkonium chloride). Nevertheless, sodium lauryl sulfate, a very irritant detergent, altered morphology and down-regulated all membrane markers. These preliminary data suggest that in vitro modulation of 33D1 expression by strong sensitizers may be an approach to the development of an in vitro model for the identification of chemicals that have the potential to cause skin sensitization and to distinguish them as far as possible from irritants.

Jepson GW, McDougal JN. **Predicting vehicle effects on the dermal absorption of halogenated methanes using physiologically based modeling.** Toxicol Sci 1999;48(2):180-8.
Occupational and environmental settings present opportunities for humans to come into contact with a variety of chemicals via the dermal route. The chemicals contacting the skin are likely to be diluted with a vehicle or present as a component of a mixture. In order to support risk assessment activities, we evaluated the vehicle effects on dermal penetration of two halogenated hydrocarbons, dibromomethane (DBM) and bromochloromethane (BCM). In vivo exposures to 15 combinations of of these in water, mineral oil, and corn oil vehicles were conducted, and blood was sampled for dibromomethane and

bromochloromethane during the exposure at 0.5, 1, 2, 4, 8, 12, and 24 h. A physiologically based pharmacokinetic (PBPK) model was used to estimate the total amounts of dibromomethane or bromochloromethane that were absorbed during the exposure, and the dermal permeability coefficients were determined. While the permeability coefficients for dibromomethane and bromochloromethane were approximately 73- and 40-fold higher, respectively, in the water vehicle than in the corn oil, the permeability coefficient, when normalized for the skin:vehicle matrix partition coefficient, varied by less than a factor of 2. The permeability in an aqueous vehicle was then successfully used to predict the permeability coefficient for dibromomethane in a nonpolar vehicle, peanut oil.

Jiang R, Benson HA, Cross SE, Roberts MS. **In vitro human epidermal and polyethylene membrane penetration and retention of the sunscreen benzophenone 3 from a range of solvents.** Pharm Res 1998 Dec;15:1863-8.
IPA COPYRIGHT: ASHP The human epidermal and polyethylene membrane penetration and retention of oxybenzone (benzophenone 3) from a range of single solvent vehicles were studied in vitro. Maximal oxybenzone fluxes from the solvents across the 2 membranes varied widely. Both the flux and estimated permeability coefficient and skin-vehicle partitioning of the compound appeared to be related to the vehicle solubility parameter. The major effects of solvents on oxybenzone flux appeared to be via changes in its diffusivity through the membranes.

Kim YO, Chung HJ, Chung ST, Kim JH, Park JH, Kil KS, Cho DH. **Phototoxicity of melatonin.** Arch Pharm Res 1999;22(2):143-50.
Melatonin (MLT), N-acetyl-5-methoxytryptamine, is mainly secreted by the pineal gland. The ultraviolet (UV), infrared (IR) and 1H-NMR spectra of irradiated and non-irradiated MLT were measured, and phototoxicity tests of MLT, anthracene (positive control) and sodium lauryl sulfate (SLS, negative control) were performed. The methods employed include both in vitro tests such as MTS assay using the human fibroblast cell and yeast growth inhibition assay using Candida albicans and in vivo method using the skin of guinea pig. UV absorption spectra and 1H-NMR spectra of MLT were changed by UVA (365 nm, 15 J/cm2), but IR spectra of MLT were not changed. The fifty percent inhibitor concentration (IC50) ratio (UV-/UV+) of MLT was 10. The inhibition zone of irradiated-paper disks treated with MLT was not observed. According to the results of histopathological examination, no pathologic lesion was observed in the non-irradiated group, but slight degeneration of keratinocytes in the epidermis, hemorrhage and vasodilation in dermis were observed in the irradiated group. These results indicate that the molecular structure of MLT is altered by UVA to unidentified photoproducts and a moderate phototoxicity of MLT is predicted.

Lin S, Xing QF, Chien YW. **Transdermal testosterone delivery: comparison between scrotal and nonscrotal delivery systems.** Pharm Dev Technol 1999;4(3):405-14.
The purpose of this investigation was to study the bioequivalence of two testosterone transdermal delivery systems (T-TDSs). Testoderm, designed to deliver testosterone through scrotal skin, and Androderm, designed for nonscrotal permeation. In vitro permeation and release kinetics as well as in vivo pharmacokinetics in the castrated Yucatan miniature swine (minipigs) model of both T-TDSs were studied side by side under the same experimental conditions. In vitro skin permeation kinetics studies demonstrated that testosterone permeates through minipig dorsal skin at zero-order kinetics from both T-TDSs. The nonscrotal T-TDS, however, has a permeation rate which is approximately 13 times higher

than that for the scrotal T-TDS. The release of testosterone from the nonscrotal T-TDS showed a biphasic release profile between cumulative amount released and time, whereas a monophasic release profile between cumulative amount released and square root of time was observed for the scrotal T-TDS. Pharmacokinetic analysis of plasma testosterone profiles in minipigs indicated a significant difference ($p < 0.001$) in daily dose of testosterone delivered (1.20 versus 4.83 mg/day), maximum concentration (Cmax) (54.2 versus 218.0 ng/dl), and area under concentration-time curve (AUC0-28) [665 versus 3208 (ng/dl) x hr] between these T-TDSs. However, there is no difference in time to reach Cmax mean residence time, and daily-delivered-dose-normalized Cmax and AUC0-28. The difference in pharmacokinetic profiles resulted from the difference in daily doses delivered, which could be attributed remarkably to the difference in permeation rate (approximately 13-fold) between the nonscrotal and scrotal T-TDSs.

Masson M, Loftsson T, Masson G, Stefansson E. **Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing**. J Controlled Release 1999 May 1;59:107-118.
IPA COPYRIGHT: ASHP The mechanism for the penetration enhancing effects of cyclodextrins was studied using hairless mouse skin or a semi-permeable membrane and a model of aqueous and lipophilic membrane diffusion. The model was described by a simple mathematical equation where properties of the system were expressed by 2 constants. Data for the permeation of a model drug through mouse skin in the presence of cyclodextrins and cyclodextrin polymer mixtures were fitted to obtain values for the 2 constants. A rise in flux with increased cyclodextrin complex concentration and fall with excess cyclodextrin was accurately predicted. Data for drug permeation through semi-permeable membrane could also be fitted to the equation. The results indicated that cyclodextrins enhance permeation by carrying drug through the aqueous barrier from bulk solution to the lipophilic surface of biological membranes.

Meidan VM, Docker MF, Walmsley AD, Irwin WJ. **Phonophoresis of hydrocortisone with enhancers: acoustically defined model**. Int J Pharm 1998 Aug 15;170:157-68.
IPA COPYRIGHT: ASHP An acoustic model was developed and used to determine the impact of ultrasound on the penetration of hydrocortisone through whole rat skin in vitro; the effects of absorption enhancers and conductive heating were also determined. Ultrasound dosimetry measurements were used to define an ultrasound source used to measure the phonophoretic enhancement of drug transport through rat skin. Acoustic dosimetry measurements indicated that the skin barrier was exposed to ultrasound standing waves and this focused heat generation with the tissue. While sonication alone did not significantly enhance hydrocortisone permeation, a significant synergistic effect was observed with laurocapram (Azone), but not with oleic acid. The ultrasound-laurocapram effect could be duplicated with conductive heating.

Merino V, Lopez A, Kalia YN, Guy RH. **Electrorepulsion versus electroosmosis: effect of pH on the iontophoretic flux of 5-fluorouracil.** Pharm Res 1999;16(5):758-61.
PURPOSE: To delineate the contributions of electrorepulsion and electroosmosis to the iontophoretic flux of 5-FU across porcine skin in vitro. Also, the isoelectric point (pI) of the skin model was determined. METHODS: The electrotransport of 5-FU, anode-to-cathode ("anodal") and cathode-to-anode ("cathodal") was determined as a function of the pH of the electrolyte bathing the skin. RESULTS: At pH 8.5, the drug (pKa approximately 8) is negatively charged and "cathodal", viz.

electrorepulsive, transport is much greater than that in the opposite direction. At pH 7.4, where approximately 25% of 5-FU is charged, electrorepulsive and electroosmotic ("anodal") fluxes are balanced. Decreasing the pH to 6, and then 5, reduces the percentage of ionized 5-FU such that "anodal" electroosmosis dominates across the negatively-charged membrane. But, at pH 4, "anodal" and "cathodal" fluxes are again equal suggesting neutralization of the skin (i.e., pI approximately 4). This is confirmed at pH 3, where "cathodal" electroosmosis dominates across the now net-positively charged barrier. CONCLUSIONS: Electrotransport is sensitive, mechanistically, to the properties of the permeant and of the skin; interactions of, for example, the drug or constituents of a formulation, that alter the barrier's net charge, can affect iontophoretic delivery. The pI of porcine ear skin is approximately 4.

Nangia A, Patil S, Berner B, Boman A, Maibach H. **In vitro measurement of transepidermal water loss: rapid alternative to tritiated water permeation for assessing skin barrier functions**. Int J Pharm 1998 Aug 1;170:33-40.
IPA COPYRIGHT: ASHP Transepidermal water loss measured with an evaporimeter was used as a rapid assessment of the integrity of the barrier properties of human skin as part of in vitro skin permeation studies using 5 categories of experimentally damaged skin models. Transepidermal water loss correlated with tritiated water permeation at short times and was found to be a rapid, convenient measurement providing a clear indication of the time dependence of barrier integrity.

Nemecek GM, Dayan AD. **Safety evaluation of human living skin equivalents.** Toxicol Pathol 1999;27(1):101-3.
Human living skin equivalents (LSEs) offer an alternative to the use of split-thickness autografts for the treatment of hard-to-heal wounds. LSEs consist of 4 active components: a well-differentiated stratum corneum derived from epidermal keratinocytes, dermal fibroblasts, and an extracellular collagen matrix. Neonatal foreskins are used as the source of keratinocytes and dermal fibroblasts for the manufacture of LSEs. Following isolation and expansion in vitro, the cells are cultured on a 3-dimensional scaffold to give an upper epidermal layer and supporting dermal layer. The resulting product has the appearance and handling characteristics of human skin. Safety evaluation of LSEs begins with insuring that foreskins are obtained only from healthy infants whose mothers are negative for a panel of adventitious agents. Keratinocyte and fibroblast cell banks are characterized using morphologic, biochemical, and histologic criteria; checked for the absence of contaminating cell types such as melanocytes, macrophages, lymphocytes, and Langerhans cells; subjected to rigorous microbiological testing (with any production materials of biological origin); and evaluated for in vivo tumorigenicity. The consistency of certain key morphologic and functional characteristics are regularly assessed. Because an LSE represents an allogeneic graft, preclinical safety studies include in vitro and in vivo determinations of its potential immunogenicity. Immunocompromised (SCID) mice reconstituted with human leukocytes or engrafted with human fetal hematolymphoid organs have been useful animal models for assessing possible immunologic responses to LSEs. Additional preclinical studies are being conducted to show that LSEs are noncytotoxic and lack allergenic, sensitizing, or irritation potential.

Perkins MA, Osborne R, Rana FR, Ghassemi A, Robinson MK. **Comparison of in vitro and in vivo human skin responses to consumer products and ingredients with a range of irritancy potential.**

Toxicol Sci 1999;48(2):218-29.
Human skin equivalent cultures were investigated as possible pre-clinical skin irritation screens to aid safety assessments for chemicals and product formulations, and to facilitate design of safe and efficient human studies. In vitro responses in human skin equivalent cultures were compared directly to in vivo human skin responses from historic or concurrent skin tests for representative chemicals and products, including surfactants, cosmetics, antiperspirants, and deodorants. The in vivo data consisted of visual scores (i.e., erythema and edema) from skin-patch tests and diary accounts of skin irritation from product-use studies. In the in vitro studies, cornified, air-interfaced human skin cultures (EpiDerm) were evaluated using methods designed to parallel human clinical protocols with topical dosing of neat or diluted test substances to the stratum corneum surface of the skin cultures. The in vitro endpoints have previously been shown to be relevant to human skin irritation in vivo, including the MTT metabolism assay of cell viability, enzyme release (lactate dehydrogenase and aspartate aminotransferase), and inflammatory cytokine expression (Interleukin-1alpha). For surfactants, dose-response curves of MTT cell-viability data clearly distinguished strongly-irritating from milder surfactants and rank-ordered irritancy potential in a manner similar to repeat-application (3x), patch-test results. For the antiperspirant and deodorant products, all the in vitro endpoints correlated well with consumer-reported irritation (r, 0.75-0.94), with Interleukin-1alpha (IL-1alpha) release, showing the greatest capacity to distinguish irritancy over a broad range. IL-1alpha release also showed the best prediction of human skin scores from 14-day cumulative irritancy tests of cosmetic products. These results confirm the potential value of cornified human skin cultures as in vitro pre-clinical screens for prediction of human skin irritation responses. A preliminary report of these results has been published.

Robinson MK, Whittle E, Basketter DA. **A two-center study of the development of acute irritation responses to fatty acids.** Am J Contact Dermatol 1999;10(3):136-45.
BACKGROUND: A human 4-hour patch test has been developed for evaluating the acute irritation potential of chemicals. This method was developed for comparative irritation assessments. Although skin irritation responses in human subjects can be quite variable, this test method has proven robust in both intra- and interlaboratory tests. However, the previous interlaboratory studies were not optimal in that slightly differing protocols were used and the studies were not controlled for time of year or source of test chemicals. As a result, some variation in acute irritation responses were seen that might have been reduced somewhat had these variables been controlled to a greater extent. OBJECTIVE: The purpose of the current study was to examine interlaboratory reproducibility of the 4-hour patch test when conducted under as identical as set of test conditions as possible. METHODS: Two laboratories conducted a direct comparison study of the acute irritation potential of three structurally related, undiluted fatty acids (octanoic acid, decanoic acid, and dodecanoic acid) in comparison to a benchmark positive control chemical (20% sodium dodecyl sulfate [SDS]). The studies were run within a 4-month period using the same commercial source of test chemicals. Test subjects were treated with each chemical under occluded patch conditions for gradually increasing exposure duration up to 4 hours. The results were then evaluated in terms of total cumulative incidence of positive responses and time response patterns. RESULTS: Using statistical comparisons of the proportion of the subjects with a positive irritant reaction to each substance, the rank order of irritation potential was decanoic acid >/= octanoic acid > SDS >> and dodecanoic acid. The statistical comparisons and the time-response patterns for each chemical were nearly identical at the two laboratories. There were also very similar, and intriguing,

variations in the interchemical response patterns seen in the two studies. CONCLUSION: When conducted under as controlled a set of test conditions as was reasonably possible, this acute irritation protocol shows remarkably high consistency across independent test laboratories, further supporting its continued development and acceptance as a valid and more predictive tool for assessing skin irritation potential.

Stark HJ, Baur M, Breitkreutz D, Mirancea N, Fusenig NE. **Organotypic keratinocyte cocultures in defined medium with regular epidermal morphogenesis and differentiation.** J Invest Dermatol 1999;112(5):681-91.
Skin equivalents formed by keratinocytes cocultured with fibroblasts embedded in collagen lattices represent promising tools for mechanistic studies of skin physiology, for pharmacotoxicologic testing, and for the use as skin substitutes in wound treatment. Such cultures would be superior in defined media to avoid interference with components of serum or tissue extracts. Here we demonstrate that a defined medium (supplemented keratinocyte defined medium) supports epidermal morphogenesis in organotypic cocultures equally well as serum-containing medium (mixture of Ham's F12 and Dulbecco's modified Eagle's medium), as documented by hallmarks of the epidermal phenotype studied by immunofluorescence and electron microscopy. In both cases regularly structured, orthokeratinized epithelia evolved with similar kinetics. Morphology in mixture of Ham's F12 and Dulbecco's modified Eagle's medium was slightly hyperplastic, and keratins 1 and 10 synthesis less co-ordinated than in supplemented keratinocyte defined medium, but a consistently inverted sequence of expression of keratins 1 and 10 was found in either medium. The late differentiation markers filaggrin, involucrin, keratin 2e, and transglutaminase 1 corresponded in their typical distribution in upper suprabasal layers. Keratin 16 persisted under both conditions indicating the activated epidermal state. Keratinocyte proliferation was comparable in both media, whereas fibroblast multiplication and proliferation was delayed and reduced in supplemented keratinocyte defined medium. In both media, ultrastructural features of epidermal differentiation as well as reconstitution of a basement membrane occurred similarly. Immature lamellar bodies and cytoplasmatic vacuoles, however, indicated an impaired lipid metabolism in supplemented keratinocyte defined medium. Nevertheless, these defined organotypic cocultures provide a suitable basis for in vitro skin models to study molecular mechanisms of tissue homeostasis and for use in pharmacotoxicologic testing.

Steiling W, Bracher M, Courtellemont P, De Silva O. **The HET-CAM, a useful in vitro assay for assessing the eye irritation properties of cosmetic formulations and ingredients.** Toxicol In Vitro 1999;13(2):375-84.
BIOSIS COPYRIGHT: BIOL ABS. One of the most important biological properties of consumer products, and also of many raw materials, is the local compatibility to mucous membranes. Until now standardized in vivo tests are accepted by public health authorities as valid to estimate the irritation potential of chemicals and suitable for the risk assessment. Nevertheless, the controversial discussion on animal tests, and particularly on the Draize rabbit eye test, is increasing in the public and scientific domain. Efforts have be try and Perfumery Association) study on alternatives to the Draize rabbit eye test are described. Furthermore, the HET-CAM test results of the finalized phase I of the above-mentioned study are discussed in detail. Prior to the COLIPA validation study, the HET-CAM was prevalidated with about 100 test substances covering a broad spectrum of chemical structures and

physical appearances and representing the range of chemicals in the cosmetics industry. This prevalidation was performed with a string dation study, testing 55 coded chemicals in four different laboratories. The HET-CAM has been established and proven to be a robust test with a good prediction of irritation potential. According to strict associations of well-defined irritation categories (in vivo and in vitro), and with the concrete PM, the in vivo irritation potential of 29 out of 55 test articles (about 52%) were correctly predicted with the HET-CAM in at least three laboratories. This quality of prediction was of different s especially of persistent slight effects on the cornea can be done properly with additional data such as physicochemical data and biological information of the test substance.

Sznitowska M, Janicki S, Williams AC. **Intracellular or intercellular localization of the polar pathway of penetration across stratum corneum**. J Pharm Sci 1998 Sep;87:1109-14.
IPA COPYRIGHT: ASHP The percutaneous penetration of baclofen, a model zwitterion, was studied in vitro using human cadaver skin to determine the intracellular or intercellular localization of the polar pathway across stratum corneum. The delipidization of skin did not result in an extremely high penetration of baclofen. The effect depended on the polarity of the solvents used, and any enhancement in penetration was observed only when skin was pretreated with solvents that extracted polar lipids. The results suggested that the polar pathway of penetration consisted of aqueous micropores localized intercellularly between the laminar lipid structures. The extraction of lipids increased the volume and number of micropores, although their tortuosity and discontinuity may not have changed. Nonpolar lipids were not primarily responsible for the formation of micropores, which probably occurred as separate domains.

Uchino T, Tokunaga H, Ando M. **ProstaglandinE2 release, squalene monohydroperoxide production and cell toxicity of skin2 ZK1301 as a human skin model in the presence of haematoporphylin ultraviolet-A irradiation.** Toxicol In Vitro 1999;13(3):483-9.
BIOSIS COPYRIGHT: BIOL ABS. For the clarification of the mechanism of ultraviolet-A (UVA)-induced cytotoxicity to skin, the prostaglandinE2 (PGE2) release and squalene monohydroperoxide (SQOOH) production in a human skin model (Skin2 ZK1301) in the presence of haematoporphylin (HP) as a photosensitizer were investigated. The PGE2 release and SQOOH production were significantly increased depending on both the irradiation time of UVA (350-380 nm) and the HP concentration. In addition, concentration-dependent inhibitions ofreen evaluations.

Vanbever R, Pliquett UF, Preat V, Weaver JC. **Comparison of the effects of short, high-voltage and long, medium-voltage pulses on skin electrical and transport properties**. J Controlled Release 1999 Jun 28;57:35-47.
IPA COPYRIGHT: ASHP The effects of electroporation using short, high-voltage or long, medium-voltage pulses on skin electrical and transport properties were studied in vitro using sulforhodamine as a model drug. While both protocols induced similar alterations and recovery processes of skin electrical resistance, long pulses were more efficient in transporting molecules across skin. Skin resistance decreased by 3 (short pulses) and 2 (long pulses) orders of magnitude, followed by incomplete recovery in both cases. For the same total transport charge, long pulses induced faster and greater transport than short pulses. A greater fraction of aqueous pathways created by the electric field was involved in molecular transport using long pulses. Transport was concentrated in localized transport regions for both protocols; fewer, but larger aqueous pathways were created by long pulses.

Wang G, Hallberg LM, Saphier E, Englander EW. **Short interspersed DNA element-mediated detection of UVB-induced DNA damage and repair in the mouse genome, in vitro, and in vivo in skin.** Mutat Res 1999;433(3):147-57.
We report a sensitive, SINE (Short Interspersed DNA Element)-mediated, PCR-based, DNA damage detection assay. Here, the SINE assay is used for detection of UVB-induced DNA damage and repair in cultured mouse cells and in vivo, in mouse skin. The unique feature of the SINE assay is its ability to support simultaneous amplification of multiple, random segments of genomic DNA. This can be accomplished due to the remarkable abundance, dispersion and conservation of SINEs in mammalian genomes. The most abundant SINEs in the mouse genome are the B1 elements, at a copy number of 50,000-80,000. Due to their strong sequence conservation, primers complementary to the B1 consensus sequence anneal to the majority of their targets in the genome. Consequently, long segments of genomic DNA located between pairs of B1 elements are efficiently amplified by PCR. Thus, in conjunction with the fact that many types of DNA adducts form blocks for thermostable polymerase, the B1 element anchored PCR makes a sensitive and versatile tool for assessing the overall integrity of the transcribed regions in mouse genome. We measured UVB-dose (0.1-3 kJ m-2) dependent formation of photoproducts in DNA from cultured cells, and after 20 h observed a substantial removal of damage at doses lower or equal to 0.6 kJ m-2. The sensitivity of detection of UVB-photoproducts formation and repair was compared to that of the conventional, single locus-targeting QPCR. Using the SINE assay we also have shown the distribution of UVB and UVC induced DNA adducts at a single nucleotide resolution within the B1 elements in mouse DNA. Lastly, we demonstrated that the sensitivity of the SINE assay is adequate for measurement of UVB-dose (1-6 kJ m-2) dependent formation and subsequent removal of photoproducts in vivo, in mouse skin.

Wang XJ, Liefer KM, Tsai S, O'Malley BW, Roop DR. **Development of gene-switch transgenic mice that inducibly express transforming growth factor beta1 in the epidermis.** Proc Natl Acad Sci U S A 1999;96(15):8483-8.
Previous attempts to establish transgenic mouse models to study the functions of transforming growth factor beta1 (TGFbeta1) in the skin revealed controversial roles for TGFbeta1 in epidermal growth (inhibition vs. stimulation) and resulted in neonatal lethality in one instance. To establish a viable transgenic model for studying functions of TGFbeta1 in the skin, we have now developed transgenic mice, which allow focal induction of the TGFbeta1 transgene in the epidermis at different expression levels and at different developmental stages. This system, termed "gene-switch," consists of two transgenic lines. The mouse loricrin vector targets the GLVPc transactivator (a fusion molecule of the truncated progesterone receptor and the GAL4 DNA binding domain), and a thymidine kinase promoter drives the TGFbeta1 target gene with GAL4 binding sites upstream of the promoter. These two transgenic lines were mated to generate bigenic mice, and TGFbeta1 transgene expression was controlled by topical application of an antiprogestin. On epidermal-specific induction of the TGFbeta1 transgene, the BrdUrd labeling index in the transgenic epidermis decreased 6-fold compared with controls. Induction of the TGFbeta1 transgene expression also caused epidermal resistance to phorbol 12-myristate 13-acetate-induced hyperplasia, with a reduction in both epidermal thickness and BrdUrd labeling compared with those in controls. In addition, TGFbeta1 transgene expression induced an increase in angiogenesis in the dermis. Given that the TGFbeta1 transgene can affect both the epidermis

and dermis, this transgenic model will provide a useful tool for studying roles of TGFbeta1 in wound-healing and skin carcinogenesis in the future.

Warren R, Sanders LM, Curtis SL, Wong LF, Zhu C, Tollens FR, Otte TE. **Human in vitro and in vivo cutaneous responses to soap suspensions: Role of solution behavior in predicting potential irritant contact dermatitis.** In Vitro Mol Toxicol 1999;12(2):97-107.
BIOSIS COPYRIGHT: BIOL ABS. Over the years a number of methods have been developed to assess the potential contact irritancy of cosmetic-related surfactants. These methods include in vitro exposure using skin equivalent cultures, proteins, enzymes, etc. These methods are exaggerated in so far as the time of exposure, concentration of material tested, and/or material composition do not represent typical use. Moreover, these in vitro data generally do not show a relationship with in vivo outcome. We present a method that uti e protocol and in vitro interleukin-1alpha excretion. Using this modified in vitro methodology and related methods, our data suggest that the irritant potential of Na-soap is largely related to the bio-availability of its Na-laurate content and interaction with the skin surface.

Wigger-Alberti W, Fischer T, Greif C, Maddern P, Elsner P. **Effects of various grit-containing cleansers on skin barrier function.** Contact Dermatitis 1999;41(3):136-40.
Products intended for individuals in contact with strongly adhering dirt often contain grit. Various clinical test methods have been developed for evaluating the potential of personal washing products to induce skin irritation. In the present study, differences in the irritant effects of washing products containing naturally-derived grit and synthetic grit were investigated in a forearm wash test. The forearms of 16 test subjects were washed in a total of 18 treatments (4 per day for 4 days, with 2 treatments on the 5th day). Treatment consisted of continuous washing for 2 min by a technician, who gently slid his fingertips with the lather up and down the forearm. Non-invasive instrumental measurements of skin barrier function were performed. Repetitive washing for 1 week lead to increased TEWL values, skin redness and decreased stratum corneum hydration. Results indicate differences in irritancy potential due to different types of grit, their surface and concentration. It is concluded that the repeated wash test seems to be adequate for rating personal washing products that contain grit.

ECOTOXICITY

Abnet CC, Tanguay RL, Hahn ME, Heideman W, Peterson RE. **Two forms of aryl hydrocarbon receptor type 2 in rainbow trout (Oncorhynchus mykiss). Evidence for differential expression and enhancer specificity.** J Biol Chem 1999;274(21):15159-66.
Two aryl hydrocarbon receptors (AhRs), rtAhR2alpha and rtAhR2beta, were cloned from rainbow trout (rt) cDNA libraries. The distribution of sequence differences, genomic Southern blot analysis, and the presence of both transcripts in all individual rainbow trout examined suggest that the two forms of rtAhR2 are derived from separate genes. The two rtAhR2s have significant sequence similarity with AhRs cloned from mammalian species, especially in the basic helix-loop-helix and PAS functional domains located in the amino-terminal 400 amino acids of the protein. In contrast, the Gln-rich transactivation domain found in the carboxyl-terminal half of mammalian AhRs is absent from both rtAhR2s. Both clones were expressed by in vitro transcription/translation and proteins of approximately 125 kDa were produced. These proteins bind 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and are able

to bind dioxin response elements in gel shift assays. rtAhR2alpha and rtAhR2beta are expressed in a tissue-specific manner with the highest expression of rtAhR2beta in the heart. Expression of rtAhR2alpha and rtAhR2beta mRNAs is positively regulated by TCDD. Both rtAhR2alpha and rtAhR2beta produced TCDD-dependent activation of a reporter gene driven by dioxin response elements. Surprisingly, the two receptors showed distinct preferences for different enhancer sequences. These results suggest that the two receptor forms may regulate different sets of genes, and may play different roles in the toxic responses produced by AhR agonists such as TCDD.

Assaf NA, Pothuluri JV, Wang RF, Cerniglia CE, Moffitt CM. **Bioassay procedure for the evaluation of erythromycin activity in aquaculture environments.** J World Aquacult Soc 1999;30(2):137-46. BIOSIS COPYRIGHT: BIOL ABS. A new bioassay procedure was developed for the detection of erythromycin in aquaculture samples using a strain of a Stenotrophomonas as an indicator organism. Conventional disk-plate and well-plate radial diffusion assay procedures were developed, as well as a third procedure using the same indicator organism in Luria-Bertani (LB) broth, supplemented with the indicator dye Brilliant Black (40 mug/mL) in a multi-well microtiter plate. For both the disk-plate and well-plate radial diffusion assays ethod allows for processing of more samples and more replication on a single titer plate. Thisnew indicator organism is specific for erythromycin when tested in the presence of other antibacterial agents, i.e., oxytetracycline (Terramycin) and/or Romet-30 . This new bioassay procedure is suitable for quantitation of low concentrations of erythromycin in aquaculture water and sediment samples.

Bengtsson BE, Bongo JP, Eklund B. **Assessment of duckweed Lemna aequinoctialis as a toxicological bioassay for tropical environments in developing countries.** Ambio 1999;28(2):152-5. BIOSIS COPYRIGHT: BIOL ABS. The tropical duckweed Lemna aequinoctialis Welwitsch (Lemnaceae) was collected in Thailand and the Philippines, so that an efficient toxicological bioassay for tropical environments could be developed. Optimal conditions required for conducting ecotoxicological growth tests were investigated and the resulting test manual was used to test the effects of heavy metals (Cd2+, Cr6+, Cu2+, Hg2+, Zn2+), ClO3-, 3,5-dichlorophenol and phenol on the growth of the two strains of duckweed. The effect on gro.

Boese BL, Ozretich RJ, Lamberson JO, Swartz RC, Cole FA, Pelletier J, Jones J. **Toxicity and phototoxicity of mixtures of highly lipophilic PAH compounds in marine sediment: can the SIGMAPAH model be extrapolated?** Arch Environ Contam Toxicol 1999;36(3):270-80. BIOSIS COPYRIGHT: BIOL ABS. The additivity of toxic units was tested using sediments contaminated with mixtures of highly lipophilic (log Kow > 4.5) parent and alkylated PAHs. The direct toxicity and photoinduced toxicity of these mixtures were examined in standard 10-day sediment toxicity tests using the infaunal amphipod Rhepoxinius abronius, with mortality and the survivors' ability to rebury as endpoints. Survivors of the initial 10-day tests were then exposed for 1 h to ultraviolet (UV) radiation and the results compa olating the Kow-LC50 QSAR or insufficient exposure duration might also have accounted for the observed results. Critical body residue (CBR) estimates for R. abronius were similar while BSAF values were much larger (10) in comparison to other studies, which used amphipods and PAHs. The phototoxicity of mixtures of contaminants were similar to the phototoxicity of single contaminants when expressed on a molar basis, which suggests that phototoxicities may be roughly additive.

Bolognesi C, Landini E, Roggieri P, Fabbri R, Viarengo A. **Genotoxicity biomarkers in the assessment of heavy metal effects in mussels: experimental studies.** Environ Mol Mutagen 1999;33 (4):287-92.
Heavy metals are stable and persistent environmental contaminants. The range of metal concentrations is generally below acute thresholds in coastal areas, where recognition of chronic sublethal effects is more relevant. Evidence of long-term adverse effects, such as cancer, due to heavy metals in marine animals comes from a number of field and experimental studies. The mechanism of metal carcinogenicity remains largely unknown, although several lines of experimental evidence suggest that a genotoxic effect may be involved. The aim of our study was to evaluate the sensitivity of genotoxicity tests, alkaline elution and micronucleus test, as biomarkers for the detection of heavy metals in mussels as the sentinel species. Experimental studies were carried out on Mytilus galloprovincialis exposed in aquarium (5 days) to different concentrations of three selected metal salts, CuCl2 (5, 10, 20, 40, 80 micrograms/l/a), CdCl2 (1.84, 18.4, 184 micrograms/l/a), and HgCl2 (32 micrograms/l/a), and to a mixture of equimolar doses of the three metals to study the results of their joint action. Metallothionein quantitation was used as a marker of metal exposure. Lysosomal membrane stability was applied to evaluate the influence of physiological status on genotoxic damage. The ranking of genotoxic potential was in decreasing order: Hg > Cu > Cd. Cu and Hg caused an increase of DNA single-strand breaks and micronuclei frequency. Cd induced a statistical increase of DNA damage, but gave negative results with the micronucleus test. A relationship between genotoxic effects and metallothionein content was observed. Reduction in lysosomal membrane stability with the increasing concentration of heavy metals was also evident. Copyright 1999 Wiley-Liss, Inc.

Burch SW, Fitzpatrick LC, Goven AJ, Venables BJ, Giggleman MA. **In vitro earthworm Lumbricus terrestris coelomocyte assay for use in terrestrial toxicity identification evaluation.** Bull Environ Contam Toxicol 1999;62(5):547-54.
BIOSIS COPYRIGHT: BIOL ABS. RRM RESEARCH ARTICLE LUMBRICUS TERRESTRIS EARTHWORM TOXICOLOGY BIOCHEMISTRY AND BIOPHYSICS COELOMOCYTE BIOASSAY COPPER SULFATE TOXIN AROCLOR 1254 PHAGOCYTOSIS IMMUNE SYSTEM BIOASSAY METHOD.

Burkhardt-Holm P, Bernet D, Hogstrand C. **Increase of metallothionein-immunopositive chloride cells in the gills of brown trout and rainbow trout after exposure to sewage treatment plant effluents.** Histochem J 1999;31(6):339-46.
Metallothionein, a biomarker of exposure and toxicity of heavy metals, has been detected in the gills of brown trout (Salmo trutta fario L.) and rainbow trout (Oncorhynchus mykiss Richardson) by means of immunohistochemistry. A very prominent labelling of chloride cells was found after exposure to diluted sewage plant effluents. No significant increase was observed in either the number of labelled cells or their labelling intensity after exposure to water of a polluted river compared to fish kept in tap water. These results do not correlate with findings of a histopathological study, suggesting that the metal levels at the sewage treatment plant were too low to produce gross histopathology. A comparison between the species indicated that the rainbow trout showed a generally higher metallothionein expression than the brown trout.

Call DJ, Liber K, Whiteman FW, Dawson TD, Brooke LT. **Observations on the 10-day Chironomus tentans survival and growth bioassay in evaluating Great Lakes sediments.** J Great Lakes Res 1999;25(1):171-8.
BIOSIS COPYRIGHT: BIOL ABS. A 10-day bioassay with larval chironomids (Chironomus tentans) was used to evaluate sediment samples from harbors at Michigan City, IN, St. Joseph, MI, Grand Haven, MI, and Toledo, OH for toxicity, based upon the endpoints of survival, dry weight, and growth. Larval responses in sediment samples from each harbor were compared to responses of larvae in reference sediments collected from or near each harbor. An inverse relationship between the number of survivors and mean organism dry weight or gr he assessment of sediment quality varied considerably for Toledo Harbor depending upon the particular reference sediment that was used for statistical comparisons.

Deziel E, Comeau Y, Villemur R. **Two-liquid-phase bioreactors for enhanced degradation of hydrophobic/toxic compounds.** Biodegradation 1999;10(3):219-33.
Two-liquid-phase culture systems involve the addition of a water-immiscible, biocompatible and non-biodegradable solvent to enhance a biocatalytic process. Two-liquid-phase bioreactors have been used since the mid-seventies for the microbial and enzymatic bioconversion of hydrophobic/toxic substrates into products of commercial interest. The increasing popularity of bioremediation technologies suggests a new area of application for this type of bioreactor. The toxicity and the limited bioavailability of many pollutants are important obstacles that must first be overcome in order to improve biodegradation processes. Two-liquid-phase bioreactors have the potential to resolve both limitations of biotreatment technologies by the enhancement of the mass-transfer rate of compounds with low bioavailability, and by the controlled delivery of apolar toxic compounds. This technology can also be useful in accelerating the enrichment of microorganisms degrading problematic pollutants. In this paper, we discuss the application of two-liquid-phase bioreactors to enhance the biodegradation of toxic/poorly bioavailable contaminants. Important microbial mechanisms involved in this type of system are described. Uptake of the substrates can be achieved by microorganisms freely dispersed in the aqueous phase and/or bound at the interface between the aqueous and the immiscible phases. Production of surface-active compounds and adhesion abilities are microbial features involved in the process. General guidelines for the design of two-liquid-phase bioreactors for biodegradation purposes are presented. Solvent selection should be established on specific criteria, which depend on the characteristics of target compound(s) and the microorganism(s) implicated in the biodegradation process. The central importance of maximizing the interfacial surface area is highlighted. The potential of this approach as an alternative to current biotreatment technologies is also discussed.

Edmunds j, mccarthy ra, ramsdell js. **Ciguatoxin reduces larval survivability in finfish.** Toxicon 1999;37(12):1827-32.
BIOSIS COPYRIGHT: BIOL ABS. Ciguatoxins are lipophilic polyether toxins which concentrate in the viscera and flesh of coral reef associated finfish (Hessel et al., 1960). In this study, we quantify the adverse effects of ciguatoxin on fish embryos by microinjection into the egg yolk of medaka (Oryzias latipis) embryos. Embryos microinjected with 0.1-0.9 pg/egg (ppb) of ciguatoxin exhibit cardiovascular, muscular, and skeletal abnormalities and those injected with higher levels (1.0-9.0 pg/egg) exhibit significantly reduced.

Elzen GW, Rojas MG, Elzen PJ, King EG, Barcenas NM. **Toxicological responses of the boll weevil (Coleoptera: Curculionidae) ectoparasitoid Catolaccus grandis (Hymenoptera: Pteromalidae) to selected insecticides.** J Econ Entomol 1999;92(2):309-13.
BIOSIS COPYRIGHT: BIOL ABS. A glass vial bioassay was used to determine the toxicity of 102 insecticides to 3 strains of the boll weevil, Anthonomus grandis grandis Boheman, ectoparasitoid Catolaccus grandis (Burks). Technical-grade samples of dimethoate, endosulfan, oxamyl, acephate, malathion, azinphos-methyl, cyfluthrin, methyl parathion, spinosad, and fipronil were used in bioassays. Three strains of C. grandis were tested: 2 in vivo-reared strains, (i.e., the In Vivo strain and the Sinaloa strain), and 1 in vitro-rear nts. In addition, malathion, the insecticide most widely used in boll weevil eradication, was significantly more toxic to the In Vitro strain than other treatments. However, it cannot be determined directly from the data which insecticides may be more toxic to C. grandis in the field. Bioassays must be refined so that risks to natural enemies can be predicted reliably. In so doing, chemical insecticides may be developed and used that may be selective (i.e., more toxic to a pest than to a benefic.

Fabacher DL, Little EE, Ostrander GK. **Tolerance of an albino fish to ultraviolet-B radiation.** Environ Sci Pollut Res Int 1999;6(2):69-71.
BIOSIS COPYRIGHT: BIOL ABS. We exposed albino and pigmented medaka Oryzias latipes to simulated solar ultraviolet-B (UVB) radiation to determine if albino medaka were less tolerant of UVB radiation than medaka pigmented with melanin. There was no difference in the number of albino and pigmented medaka that died during the exposure period. Spectrophotometric analyses of the outer dorsal skin layers from albino and pigmented medaka indicated that, prior to exposure, both groups of fish had similar amounts of an apparent colo.

Ferrari B, Radetski C, Veber AM, Ferard JF. **Ecotoxicological assessment of solid wastes: A combined liquid- and solid-phase testing approach using a battery of bioassays and biomarkers.** Environ Toxicol Chem 1999;18(6):1195-202.
BIOSIS COPYRIGHT: BIOL ABS. Municipal solid waste incinerator bottom ash (MSWIBA) was used as a test matrix for comparing the responses of different variables (solid phase vs leachate media) through toxicity tests and resulting endpoints. Toxicity of leachate and solid-phase MSWIBA was evaluated with three terrestrial plants, namely oats (Avena sativa L.), Chinese cabbage (Brassica campestris L. cv. chinensis), and lettuce (Lactuca sativa L.). Assessment endpoints for these plant tests were biomass fresh weight, germinatio y of MSWIBA was revealed by the solid-phase approach, whereas no toxicity was observed with the MSWIBA leachate. The increase of oxidant stress enzyme activities was demonstrated to be a good indicator of solid or leachate phase toxicity.

Fragoso NM, Hodson PV, Kozin IS, Brown RS, Parrott JL. **Kinetics of mixed function oxygenase induction and retene excretion in retene-exposed rainbow trout (Oncorhynchus mykiss).** Environ Toxicol Chem 1999;18(10):2268-74.
BIOSIS COPYRIGHT: BIOL ABS. The polycyclic aromatic hydrocarbon 7-isopropyl-1-methylphenanthrene (retene) induces mixed function oxygenase (MFO) activity of fish. Bile levels of retene and its metabolite(s) were measured in relation to exposure time, exposure concentration, and induction of MFO activity. Synchronous fluorescence spectrometry provided a rapid means of

measuring the amount of retene present in the bile of exposed fish, whereas conventional fluorescence spectrometry was used to quantify the amount of retene m Transfer of fish to clean water after 48 h of exposure resulted in a rapid decrease in the presenceof retene and its metabolite(s) in the bile, with a calculated half-life of about 14 h. In vitro additions of retene directly to the ethoxyresorufin O-deethylase assay demonstrated that retene is capable of acting as a competitive inhibitor. Thus, retene contamination of postmitochondrial supernatant (S9 fraction) could result in false-negative results in the MFO assay. The MFO activity in extrahe.

Gellert G, Stommel A, Trujillano A. **Development of an optimal bacterial medium based on the growth inhibition assay with Vibrio fischeri.** Chemosphere 1999;39(3):467-76.
BIOSIS COPYRIGHT: BIOL ABS. Chronic toxicity level of chemicals to bacteria can depend on composition and concentration of medium ingredients. This was demonstrated by means of a growth rate inhibition test with the marine bacterium V. fischeri. In a minimal medium (following the validity criterion of achieving a least cell multiplication rate) containing only yeast extract as organic nutrient component, V. fischeri was to Cu2+ 11,9 times, to Hg2+ 3 times and to Zn2+ 2,8 times more sensitive than in the complete defined me.

Goka K. **Embryotoxicity of zinc pyrithione, an antidandruff chemical, in fish.** Environ Res 1999;81 (1):81-3.
Early-life-stage toxicity tests of zinc pyrithione (Zpt) and commercial shampoos containing or not containing Zpt were performed on zebra fish and Japanese Medaka. The results showed that Zpt induced significant teratogenic effects on larvae of both species of fish. The two antidandruff shampoos containing Zpt induced the same teratogenic effects as Zpt. The calculated EC50 concentrations of Zpt for each of the shampoos were consistent with the EC50 for Zpt concentrate. Furthermore, 23 other shampoos not containing Zpt showed no teratogenic effects. These results strongly suggest that Zpt is the main factor in the antidandruff shampoos. Copyright 1999 Academic Press.

Gopalan HN. **Ecosystem health and human well being: the mission of the international programme on plant bioassays.** Mutat Res;426(2):99-102.
BIOSIS COPYRIGHT: BIOL ABS. In a broad sense, since humans are ultimately a part of the ecosystem, we may conclude that ecosystem health encompasses human health. A preventative measure that detects the environmental hazards that infringe on human health should be established on a global scale. Plant bioassays, which are most sensitive in detecting genotoxicity of environmental agents, can serve as the first alert for the presence of environmental hazards in water, air, and soil the essential elements of life. Three plant testing of the genotoxicity of environmental pollutants using these three bioassays since 1996 after the hands-on workshop conducted in Qingdao, China. The general aim of this programme was not only to identify the substance and situations that may entail a significant risk to humans as proposed by ICPEMC and IAEMS but also by using these single, quick, and inexpensive plant bioassays to demonstrate the effects of pollution and to carry on environmental education to the general public at an earl.

Gray MA, Metcalfe CD. **Toxicity of 4-tert-octylphenol to early life stages of Japanese medaka (Oryzias latipes).** Aquatic Toxicol 1999;46(2):149-54.
BIOSIS COPYRIGHT: BIOL ABS. Japanese medaka (Oryzias latipes) were exposed to octylphenol

(OP) during embryolarval life stages, from fertilization to swim-up, to determine lethal and sublethal toxicity. Replicate embryotoxicity tests produced LC50's of 450, 830, and 940 mug l-1 OP. Developmental abnormalities observed in embryos and larvae ranged from circulatory problems to failure to inflate swim bladders. The mean duration to hatch was not affected by exposure to octylphenol. Hatching success was reduced by OP exposure.

Hamza-Chaffai A, Amiard JC, Cosson RP. **Relationship between metallothioneins and metals in a natural population of the clam Ruditapes decussatus from Sfax coast: a non-linear model using Box-Cox transformation.** Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1999;123(2):153-63.
BIOSIS COPYRIGHT: BIOL ABS. Cadmium, copper and zinc were determined concomitantly with metallothionein-like proteins (MTLPs) in the subcellular fractions of Ruditapes decussatus digestive gland. This study covered 4 months and aimed to evaluate the effect of metal pollution and other factors such as sex, size and reproductive state on MTLP levels. Copper concentrations did not vary with months, however Cd and Zn concentrations showed high levels during August. Organisms showing low cadmium concentrations presented the hig nd the studied metals. A model including sex and size showed that these two factors affected MTLPlevels, but were less important than metals. Males of R. decussatus showed higher significant correlations between MTLP levels and cadmium than females. Moreover, the effect of size and reproductive state on MTLP levels was less perceptible in males than in females. As a result, MTLPs in males of R. decussatus could be proposed as suitable biomarker for detecting metal contamination.

Hartwell SI. **Empirical assessment of an ambient toxicity risk ranking model's ability to differentiate clean and contaminated sites.** Environ Toxicol Chem 1999;18(6):1298-1303.
BIOSIS COPYRIGHT: BIOL ABS. Ambient toxicity results were used to investigate statistical implications of sampling design options for an existing toxicological risk ranking model. A battery of water column and sediment toxicity bioassays measuring lethal and sublethal endpoints was employed with fish, invertebrates, vascular plants, and bacteria. Bioassays were conducted monthly from July through September 1995 with water from three stations in the South River estuary, Maryland, USA, and a reference station. Sediment bioas stream station in the South River were comparable to the reference station. Statistical analyses demonstrated that the risk ranking model does not require field sample replication for tributary-wide assessment. Characterization of an entire estuary does require broad coverage to assess the system as a whole, however. Threshold levels of toxic impact can be quantified with the model.

Hatch AC, Burton GA Jr. **Sediment toxicity and stormwater runoff in a contaminated receiving system: consideration of different bioassays in the laboratory and field.** Chemosphere 1999;39 (6):1001-17.
Several field and laboratory assays were employed below an urban storm sewer outfall to define the relationship between stormwater runoff and contaminant effects. Specifically, two bioassays that measure feeding rate as a toxicological endpoint were employed in the field and in the laboratory, along with bioassays measuring survival and growth of test organisms. In 7 to 10 d in situ exposures, amphipod leaf disc processing, growth and survival were monitored. Different exposure scenarios were investigated by varying the mesh size (74 microns or 250 microns mesh) and method of deployment

(water column, sediment surface, or containing sediment) of in situ exposure chambers. Hyalella azteca, Daphnia magna, and Pimephales promelas survival were monitored in 48 h in situ exposures. Feeding inhibition was investigated via enzyme inhibition of H. azteca and D. magna and via leaf disc processing measurements of the detritivore H. azteca. Additionally, we investigated the extent of phototoxicity at this site via field exposures in sun and shade and laboratory exposures with and without UV light. The measurement of detritivore leaf disc processing, and thus its usefulness as an endpoint, was hindered by individual variability in the amount of leaf consumed and by leaf weight gain during the summer field exposures. For D. magna, enzyme inhibition measured in a laboratory exposure did not reveal the toxicity observed in field exposures. For H. azteca, enzyme inhibition measured in the laboratory indicated toxicity similar to that observed in short term chronic in situ exposures. Enzyme inhibition also did not detect differences in toxicity due to variations in flow conditions. There were no statistically significant effects of any exposure on P. promelas survival or H. azteca growth, and there were no statistically significant effects due to mesh size or sun exposure. Survival of H. azteca was the most sensitive and the least variable endpoint. Effects on survival were noted in the same treatments over short-term chronic exposures in the laboratory and in situ. Significant differences in survival were noted due to the method of deployment under low flow conditions. In situ chambers containing sediment resulted in greater mortality in the 10 d low flow in situ experiments. Under high flow conditions, significant reductions in survival and leaf disc processing were noted under all methods of deployment at the two impacted sites over a 7 d exposure. Also under high flow conditions, significantly greater mortality of H. azteca was reported at the downstream field site when sediment was included in the chamber at deployment. These results suggest that significant toxicity at this site is due to accumulation of contaminants in the sediment and the mobilization of these contaminants during a storm event. In situ exposures detected toxicity not observed in laboratory exposures. These results suggest that a combination of laboratory and field bioassays is most useful in defining field effects.

Jung K, Kaletta K, Segner H, Schueuermann G. **15N metabolic test for the determination of phytotoxic effects of chemicals and contaminated environmental samples.** Environ Sci Pollut Res Int 1999;6(2):72-6.
BIOSIS COPYRIGHT: BIOL ABS. A stable isotope 15N-nitrogen test (ESIMA = Ecotoxicological Stable Isotope Metabolic Assay) was developed to assess biological effects and the potential toxicological hazard of chemicals and contaminated environmental samples on plant metabolism. The assay measures the effect of toxicants on the incorporation of a 15N labelled tracer into the total nitrogen fraction (both the non-protein and protein fraction) of plants. Segments of Pisum arvense epicotyls are used as test substrates because of samples. The specificity and sensitivity of effects as indicated by ESIMA were compared with effects as measured by two established ecotoxicological bioassays, the pollen tube growth test using pollen of Nicotiana sylvestris and the bacterial luminescence inhibition test using pollen of Photobacterium phosphoreum. The results of the study clearly indicate the suitability of ESIMA for assessing toxic impacts on plant nitrogen metabolism.

Kerr DS, Briggs DM, Saba HI. **A neurophysiological method of rapid detection and analysis of marine algal toxins.** Toxicon 1999;37(12):1803-25.
BIOSIS COPYRIGHT: BIOL ABS. We have examined the effectiveness of the in vitro rat hippocampal slice preparation as a means of rapidly and specifically detecting the marine algal toxins saxitoxin,

brevetoxin, and domoic acid and have identified toxin-specific electrophysiological signatures for each. Brevetoxin (PbTX3, 50-200 nM) produced a significant reduction in orthodromic population spike amplitude which was quick to reverse during a 50 min wash-out, while antidromic population spikes and field EPSPs exhibited only sl ffect on either antidromic or fibre spikes. Fifty nanomolar saxitoxin (PSP) abolished all responsesin all slices. Only antidromic spikes showed any recovery during wash-out. Field EPSP and fiber spike analysis further demonstrated that the preparation is capable of reliably detecting saxitoxin in a linearly responsive fashion at toxin concentrations of 25-200 nM, and tests of naturally contaminated shellfish confirmed the utility of this assay as a screening method for PSP. Our findings suggest.

Khangarot BS, Rathore RS, Tripathi DM. **Effects of chromium on humoral and cell-mediated immune responses and host resistance to disease in a freshwater catfish, Saccobranchus fossilis (Bloch).** Ecotoxicol Environ Saf 1999;43(1):11-20.
The effects of subtoxic levels of Cr on humoral and cell-mediated immune responses, blood parameters, susceptibility to bacterial (Aeromonas hydrophila) infection, and macrophage activity in the freshwater air-breathing Asian catfish, Saccobranchus fossilis, during a 28-day exposure were examined by a static bioassay test procedure. At 0.1, 1.0, and 3.2 mg / liter Cr, dose-dependent Cr accumulation in kidney, liver, and spleen was observed at the end of the experiment. Chromium exposure caused a significant change in spleen to body weight ratio. Fish exposed to Cr concentrations had lower antibody titer values, reduced numbers of splenic and kidney plaque-forming cells, and higher counts of splenic lymphocytes but reduced counts of kidney cells when compared with the control group. At 0.1, 1.0, and 3.2 mg /liter Cr, dose-dependent decreases in red blood cell counts, hemoglobin content, and packed cell volume were observed. Differential leukocyte counts revealed that Cr exposure caused a significant decrease in large and small lymphocytes, whereas neutrophils and thrombocytes increased. Effects of Cr exposure to mitogen (Con A) on proliferation of splenic and pronephric lymphocytes suggests a decrease in mitogenic response. The eye-allograft rejection time, as a parameter of cell-mediated immunity, was statistically increased at 1.0 and 3.2 mg/liter Cr. Fish exposed to Cr for 28 days exhibited higher susceptibility to A. hydrophila infection than control fish. The results suggest that Cr exposure reduced the resistance of catfish to bacterial infections. The phagocytic activity of splenic and pronephros macrophages was examined in vitro and found to be significantly decreased. Copyright 1999 Academic Press.

Larson JM, Karasov WH, Sileo L, Stromborg KL, Hanbidge BA, Giesy JP, Jones PD, Tillitt DE, Verbrugge DA. **Reproductive success, developmental anomalies, and environmental contaminants in double-crested cormorants (Phalacrocorax auritus).** Environ Toxicol Chem 1996;15(4):553-9.
To test an association between environmental contaminants and the prevalence of congenital anomalies in colonial waterbirds, we collected representative eggs for chemical analysis from double-crested cormorant nests at colonies in Lake Michigan, Wisconsin, USA, and Lake Winnipegosis, Manitoba, Canada, and periodically revisited the nests to determine the hatching success, survivorship of hatchlings, and number of deformed hatchlings in the remainder of each clutch. Total concentrations of polychlorinated biphenyls (PCBs) in eggs were determined by capillary gas chromatography. The combined activity of planar chlorinated hydrocarbons (PCHs) in the eggs was measured in an in vitro

bioassay based on the induction of ethoxyresorufin-O-deethylase (EROD) activity in rat hepatoma cells. The combined EROD induction activity was expressed as 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ). Total concentrations of PCBs and TCDD-EQ were seven to eight times greater in eggs from Lake Michigan (7.8 ug/g and 138 pg/g, respectively) than in those from Lake Winnipegosis (1.0 ug/g and 19 pg/g, respectively). The proportion of eggs hatching at the Lake Michigan colony (59%) was less (p less than 0.05) than at Lake Winnipegosis (70%), and the prevalence of hatchlings with deformed bills was greater (p less than 0.001) at Lake Michigan (0.79 vs. 0.06%). However, within the Lake Michigan colony, concentrations of PCBs and TCDD-EQ were not correlated with either hatching success or the occurrence of deformities in nestlings.

Layton AC, Gregory B, Schultz TW, Sayler GS. **Validation of genetically engineered bioluminescent surfactant resistant bacteria as toxicity assessment tools.** Ecotoxicol Environ Saf 1999;43(2):222-8.
Bacteria are useful organisms for measuring acute and chronic toxicity. The most popular toxicity tests utilize the inhibition of bioluminescence as an indication of toxicity. An extensive toxicity database on pure chemical compounds has been created using the bioluminescent microorganism, Vibrio fischeri. However, the use of the Microtox assay in applications for environmental samples is not always successful, due to the test organism. Because the genes for bioluminescence have been cloned from V. fischeri, environmentally relevant test strains can be readily constructed. In this study, surfactant-resistant bioluminescent bacterial strains were constructed by transferring a broad host range plasmid containing the bioluminescent genes under the regulation of a constitutive promoter into strains from several bacterial genera. Two test strains, Stenotrophomonas 3664 and Alcaligenes eutrophus 2050, were approximately 400 times more resistant to the nonionic surfactant polyoxyethylene 10 lauryl ether than V. fischeri and are useful for toxicity reduction evaluations of remediation processes which use surfactants for solubilization of hydrophobic pollutants. The use of these strains as alternative test organisms in the Microtox assay was evaluated using nonpolar narcosis as the baseline toxicity mechanisms. The two test strains and V. fischeri indicated linear fits of EC50 values with the octanol/water partition (Kow) for five nonpolar narcotic compounds in acute assays ($r2>0.9$) with a slope of approximately 1. For all three strains, the y-intercept values were approximately the same, indicating that sensitivity did not vary. These results indicate that the nonpolar narcosis baseline toxicity mechanism may be useful as a general tool to validate the functioning of genetically engineered bioluminescent microorganisms.

Lin YJ, Karuppiah M, Shaw A, Gupta G. **Effect of simulated sunlight on atrazine and metolachlor toxicity of surface waters.** Ecotoxicol Environ Saf 1999;43(1):35-7.
Atrazine and metolachlor are the two most widely used herbicides in the United States; through non-point-source runoff both herbicides may cause toxicity to aquatic organisms. Toxicity changes were measured for atrazine and metolachlor in surface waters after exposure to simulated sunlight (0, 20, and 40 kJ/m2) using a Xenon Weather-Ometer. A Microtox toxicity test, using the marine luminescent bacterium Vibrio fischeri, was conducted on deionized, river, and bay water samples mixed with atrazine or metolachlor herbicide (12 mg/liter) after exposure to simulated sunlight. Microtox test (EC50%) results demonstrated that the toxicity decreased with increasing light intensity for both herbicides in river and bay water. These results also indicate that the toxicity of the bay water, with high concentrations of organic and suspended matter, was reduced, for both herbicides, compared with the

toxicity of the river water, possibly through photodegradation of pesticides. Copyright 1999 Academic Press.

Lotufo GR, Fleeger JW. **Effects of sediment-associated phenanthrene on survival, development and reproduction of two species of meiobenthic copepods.** Marine Ecol Prog Ser 1997 May;151(1-3):91-102.
The lethal and sublethal toxicity of phenanthrene (a polycyclic aromatic hydrocarbon, PAH) to 2 species of meiobenthic estuarine harpacticoid copepods (Schizopera knabeni and Nitocra lacustris) was investigated. Individuals of different life stages (nauplius, copepodite, adult male and female) were exposed to sediment-associated phenanthrene in separate 10 d bioassays. Overall, N. lacustris (10 d LC50 values ranging from 43 to 105 ug/g dry wt) was more sensitive than S. knabeni (10 d LC50 values ranging from 84 to 349 ug/g dry wt). Significant differences in life-stage-specific sensitivity were observed for S. knabeni, with the nauplii being most sensitive, followed by copepodites, and adults; adult males and females were equally sensitive. For N. lacustris, females were significantly more sensitive than all other stages; no significant differences were evident among the other stages. Phenanthrene effects on offspring production were investigated in the adult 10 d bioassay. Significant decreases in offspring production occurred at sublethal concentrations for S. knabeni (as low as 22 ug/g dry wt), but at concentrations in the same range as the 10 d LC50 values for N. lacustris. In addition, phenanthrene significantly prolonged embryonic and larval development and decreased egg hatching success for both species. Our results suggest that PAHs have a negative effect on the reproduction of meiobenthic copepods at sublethal concentrations mostly due to a decrease in brood production rate and impairment of hatching. Overall, deleterious effects were manifested in the same range of concentrations for both species, but definite species-specific differences in the pattern of responses were evident.

Middaugh DP, Beckham N, Fournie JW, Deardorff TL. **Evaluation of bleached kraft mill process water using Microtox(R), Ceriodaphnia dubia, and Menidia beryllina toxicity tests.** Arch Environ Contam Toxicol 1997 May;32(4):367-75.
To determine whether a 7- to 10-d embryo toxicity/teratogenicity test with the inland silverside fish, Menidia beryllina, is a sensitive indicator for evaluation of bleached kraft mill effluents, we compared this test with the Microtox(R) 15-min acute toxicity test and the Ceriodaphnia dubia 7-d chronic toxicity test. Water samples used in each test were collected from three areas in a bleached kraft pulp and paper mill using a 100% chlorine dioxide bleaching process: 1) river water prior to use in the mill; 2) the combined acid/base waste stream from the pulping process prior to biological treatment in the aerated stabilization basin (ASB); and 3) the effluent from the ASB with a retention time of approximately 11 d. Relative toxicity determined by the three tests for each water sampling location was compared. All three toxicity tests were predictive indicators of toxicity; however, the C. dubia and M. beryllina tests were the more similar and sensitive indicators of toxicity. Process water (ASB influent) prior to biological treatment in the ASB was toxic at all concentrations using the Microtox(R) and C. dubia tests. The fish embryo test showed no toxicity at 1% concentrations, slight toxicity at 10%, and acute toxicity at the 100% ASB influent concentration. Tests with biologically-treated ASB effluent indicated a substantial reduction in observed toxicity to Microtox(R) bacteria, C. dubia, and M. beryllina. No toxic responses were observed in any test at a 1% ASB effluent concentration which was the approximate effluent concentration in the receiving river following mixing. No relationship was found among any

toxicological response and effluent levels of adsorbable organic halides, polychlorinated phenolic compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran, total suspended solids, color, chemical oxygen demand, or total organic carbon.

Ohta T, Tokishita S, Shiga Y, Hanazato T, Yamagata H. **An assay system for detecting environmental toxicants with cultured cladoceran eggs in vitro: malformations induced by ethylenethiourea.** Environ Res 1998 Apr;77(1):43-8.
An in vitro assay system using cladoceran eggs was developed for the detection of toxic compounds in the aquatic environment. Parthenogenetic eggs of Daphnia magna were removed from the brood chambers of female adults and cultured individually in 96-well microtiter plates with Elendt M7 medium at 23 degrees C. Embryonic development proceeded completely in vitro with more than 95% hatchability. Egg development time in vitro was 2 days, which was almost equal to that in the brood chamber of the mother. Ethylenethiourea, a compound teratogenic to mammals, was investigated for toxicity to development of eggs. Isolated eggs were cultured in the presence of ethylenethiourea during the period of embryonic development for 3 days. Treatment with ethylenethiourea induced morphological abnormalities in the cladoceran carapace. Parthenogenetic eggs of Cladocera are genotypically identical, which makes them useful biological materials for a toxicity test on aquatic pollutants.

Pagano G, Iaccarino M, Guida M, Manzo S, Melluso G, Oral R, Romanelli R, Rossi M. **Cadmium toxicity in spiked sediment to sea urchin embryos and sperm.** Marine Environ Res 1996;42(1-4):54-5.
This study was to evaluate the toxicity of cadmium to sea urchin embryogenesis and fertilization when cadmium was dissolved in seawater (sw), or in a sediment matrix. Mediterranean sea urchins (Sphaerechinus granularis and Paracentrotus lividus) were utilized in bioassays with the following toxicity endpoints: (a) developmental defects; (b) changes in fertilization success; and (c) cytogenetic abnormalities. Sediment samples were collected at one freshwater (V0) and one coastal site (V7) known to be unaffected by any major pollution sources and analyzed for organic, inorganic, and microbial contaminants. Reference sediment was sampled within two weeks before bioassays, and was spiked with CdSO4 (wt/dry wt) at different time intervals (three to ten days) before experiments. Spiked sediment (ss) was kept at 4 degrees C in sealed, air-stripped vials. Reference sediment samples, when tested at levels ranging from 0.1 to 1% (dry wt/vol), failed to show any damage to embryogenesis; fertilization was slightly inhibited by V7 samples, whereas site V0 displayed no spermiotoxic effect, or even an increase in fertilization success (hormesis). When Cd(II)-induced developmental toxicity was tested in water-only (w-o) vs ss (0.5%), at nominal Cd(II) levels ranging from 10(-8) to 5 x 10(-4) M, developmental defects were significantly increased by 10(-5) M Cd(II) w-o, whereas Cd(II)-ss only displayed a significant increase in developmental defects at the nominal concentration of 10(-4) M. Fertilization success was affected by Cd(II) following a dose-response trend, with hormesis at 10(-7) M and inhibition at levels greater than 10(-5) M Cd(II); the same trend was observed in both w-o and ss schedules. The induction of transmissible damage to the offspring of Cd(II)-exposed sperm, as well as the induction of cytogenetic aberrations in exposed embryos have been observed recently in w-o experiments, and currently subjected to confirmation in ss schedules. Altogether, the data point to an overall superimposability of Cd(II)-induced toxicity in w-o exposure vs ss in spermiotoxicity bioassays.

The observed decrease in Cd(II) toxicity in the embryos exposed to ss vs w-o might possibly reflect a loss of Cd(II) bioavailability in ss throughout the 72-h long embryogenesis, related to the late embryotoxic effect of cadmium (Pagano et al., 1982).

Raabe F, Wichmann G, Dautzenberg D, Lierse C, Zluticky J, Metzner G, Muecke W. **[To the genotoxicity of stack gas condensates of Bavarian waste incineration plants: III. Emission monitoring with a simple UDS assay using the human lung cell lines NCI-H 322 and 358]**. Zentralbl Hyg Umweltmed 1999;201(6):513-30. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. For the validation of the genotoxicity testing on stack gas condensates from waste incineration plants using bacterial short time tests (15), a modified UDS assay with the lung cell lines NCI-H 322 and 358 was developed. The UDS assay is more sensitive than the SOS chromotest and discriminates better between the negative or weakly positive and the clearly positive samples. It has a high sensitivity and specificity and also accuracy, is practicable in a comparatively simple, speedy and reasonably he two test cell lines. From three plants examined continuously in this period only two emitted stack gases with constantly low genotoxicity at the end of sampling. 5 clean gas condensates, that were taken in random samples from 3 other plants in the period 1994 to 1995, proved to be non-genotoxic in the UDS assay. However, one of these plants emitted stack gases with high cytotoxicity, which might have masked UDS-inducing single substances. It is not possible to make a statement on the human to gas emissions are necessary.

Randerath K, Randerath E, Zhou GD, Supunpong N, He LY, McDonald TJ, Donnelly KC. **Genotoxicity of complex PAH mixtures recovered from contaminated lake sediments as assessed by three different methods.** Environ Mol Mutagen 1999;33(4):303-12.
Although human exposure generally occurs to mixtures of chemicals, limited toxicological information is available to characterize the potential interactions of the components of environmental mixtures. This study was conducted to compare the genotoxicity of chemically characterized polycyclic aromatic hydrocarbon (PAH) mixtures using in vitro and in vivo techniques. A total of three extracts (E1-E3) were selected from sediment samples collected from a lake adjacent to an abandoned coal gasification site. Sediments were collected on a grid moving downstream and away from the most likely source of PAH contamination, with E1 collected closest to the shore, E2 at an intermediate distance, and E3 furthest from the shore. The sediment samples were extracted in methylene chloride and methanol, dried, and redissolved in an appropriate solvent for evaluation in a battery of genotoxicity assays. Samples were evaluated for their ability to produce point mutations in bacteria and DNA adducts in vitro without metabolic activation or in vivo. Samples were also analyzed using GC/MS. Sample E1 had both the highest concentration of benzo(a)pyrene (BP) (46.5 ppm) and carcinogenic PAHs and, using 32P-postlabeling, induced the highest adduct levels overall in vitro and in vivo. Sample E2, which had a BP concentration of 14 ppm, induced the greatest number of revertants in the bacterial mutagenicity assay. Sample E3, which had the lowest level of carcinogenic PAHs and BP, induced the lowest adduct levels. However, E3 was capable of inducing a positive genotoxic response in bacteria (with S9), although the slope of the response at lower doses was less than that of E2. The in vivo data showed that the major adduct formed by E1 and E2 was a BP adduct. This information could not have been obtained with the Salmonella or in vitro postlabeling tests. Among internal organs, the extracts of all three samples induced the greatest adduct levels in the lung, similarly to previous complex PAH mixtures studied.

These data demonstrate the limitations of predicting genotoxic or carcinogenic potential based on chemical analysis or a single biological test. The results suggest that mixture interactions, cytotoxicity and metabolism are likely to have an influence on the potential of a complex mixture of chemicals to produce a carcinogenic effect. In addition, the concentration of genotoxic PAHs and both in vitro and in vivo DNA adduct formations were decreased with increasing distance from the shoreline. Copyright 1999 Wiley-Liss, Inc.

Renoux AY, Millette D, Tyagi RD, Samson R. **Detoxification of fluorene, phenanthrene, carbazole and p-cresol in columns of aquifer sand as studied by the Microtox assay.** Water Res 1999;33 (9):2045-52.
BIOSIS COPYRIGHT: BIOL ABS. The changing in the toxicity of mixtures of creosote-related compounds during their aerobic biodegradation was studied in columns of saturated aquifer material. The Microtox assay was used to measure the toxicity of phenanthrene, fluorene, carbazole and p-cresol, added individually or in a mixture and to evaluate their detoxification during migration through the columns. When tested alone, phenanthrene, fluorene and p-cresol, inhibited Vibrio fischeri luminescence emission (15 min IC50 = 140, 7 through the columns since the inhibition caused by the effluent solutions averaged 6%. Additionally, p-cresol (10,000 mug/L) enhanced the efficiency of detoxification of the column system; the inhibition by the compound mixture decreased from 81.4% (SD = 2.0) to 15.8% (SD = 1.8) after having passed through the first 3.8 cm of the column, and was undetectable at the two other downgradient ports. A comparison of the data of product disappearance and changes in toxicity indicated that detoxificati.

Reynoldson TB, Rodriguez P, Madrid MM. **A comparison of reproduction, growth and acute toxicity in two populations of Tubifex tubifex (MSuller, 1774) from the North American Great Lakes and Northern Spain.** Hydrobiologia 1996 Oct;334(1-3):199-206.
Reproduction in Tubifex tubifex is being used as part of a suite of indicators of sediment toxicity in Canada and Spain, and reproduction of T. tubifex is being considered as a component of sediment objectives for environmental regulation and clean-up in the Canadian Great Lakes. The data being used to set these reproductive targets have been developed from a single culture of T. tubifex from Lake Erie. The plasticity of this particular species is well known and before it can be adopted widely as a test organism it is necessary to determine whether a single culture source should be used or if cultures derived from different populations respond similarly. A series of experiments with two cultures, one from Lake Erie the second from a small mountain stream in Northern Spain have shown that the Spanish worms appear to produce fewer cocoons per adult (mean 8.6 S.D. 1.0) than those from Lake Erie (mean 10.4 S.D. 0.3) at 22.5 degrees C, a standard test temperature. The number of young produced per adult by the Spanish culture is also lower (mean 19.0 S.D. 4.6) than the L. Erie population (mean 30.6 S.D. 2.3), however, the Spanish population has higher reproductions rates at a lower temperature. The Spanish worms also have lower and more variable growth rates than the Canadian population. There also appear to be slight differences in the sensitivities to toxicants, with the Canadian worms having higher LC50s for copper, chromium and cadmium. While there are differences in the responses in the two cultures these are not considered to be sufficient to invalidate the use of either population in a standard bioassay protocol as long as appropriate calibration and validation are undertaken.

Roney N. **Assessing the accuracy of the weight-of-evidence methodology in determining**

interactions of a three-component mixture. Environ Epidemiol Toxicol 1999;1(2):183-92.
BIOSIS COPYRIGHT: BIOL ABS. With the increasing number of chemicals present in our environment, there are many more opportunities for exposures to chemical mixtures. Several approaches to evaluating the risk associated with chemical mixtures have been proposed. This paper evaluates the weight-of-evidence approach to assessing interactions in chemical mixtures, as developed by Mumtaz and Durkin (Mumtaz M.M. and Durkin P.R. A weight-of-evidence scheme for assessing interactions in chemical mixtures. Toxicol. Ind. Health 1992 t many of the interactions seen in the experimental study. Although more research is needed in thefield of mixture risk assessment and in the refinement of the weight-of-evidence approach, this project lends support to using this approach when assessing concurrent exposures to multiple substances when there is a lack of experimental studies.

Sanderson JT, Commandeur JN, Van Wezel A, Vermeulen NP. **Bioassays for the detection of chemicals that can form bioactivation-dependent reactive free radicals.** Environ Toxicol Chem 1999;18(6):1236-43.
BIOSIS COPYRIGHT: BIOL ABS. In vitro bioassays were developed for the detection of chemicals that can be bioactivated to reactive free radical species in microsomal fractions. Two methods were deployed, a down-scaled spectrophotometric method for the detection of chemicals that can cause lipid peroxidation using the measurement of thiobarbituric acid-reactive substances (TBARS) and a fluorometric method for the detection of chemicals that can undergo redox cycling to generate superoxide radicals based on the detection of h t and about 100-fold lower for the potent redox cycler 2,3,5,6-tetramethylbenzoquinone (TMBQ). Several binary mixtures of chemicals were tested for potential nonadditive effects in both in vitro systems. Some antagonistic effects among halogenated methanes were observed in the lipid peroxidation assay. In the hydrogen peroxide production assay, greater than additive effects were seen between small concentrations of paraquat and TMBQ. A number of surface water concentrates from several locations rates in the hydrogen peroxide production assay and their acute toxicity in Daphnia magna. No correlation was observed between this bioassay response and toxicity in the Microtox assay using Photobacterium phosphoreum.

Scott-Fordsmand JJ, Weeks JM, Hopkin SP. **Toxicity of nickel to the earthworm and the applicability of the neutral red retention assay.** Ecotoxicol 1998;7(5):291-5.
BIOSIS COPYRIGHT: BIOL ABS. The toxic effects of nickel on survival, growth, and reproduction of Eisenia veneta were investigated following 4 weeks of exposure to a nickel-chloride spiked loamy sand soil. The ability of a simple earthworm biomarker, the lysosomal membrane stability of coelomocytes, to reflect nickel exposure was also studied. Nickel caused a significant toxic effect on E.veneta at soil concentrations above 85 mg Ni/kg. Reproduction (cocoon production) was the most sensitive parameter being reduced at soil strated a dose-response relationship. The neutral-red retention time showed large individual variation for the earthworms within each exposure concentration. It was concluded that the lysosomal membrane stability, measured as neutral red retention time, has a potential role in risk assessment, but care should be taken conducting this test.

Sibley PK, Benoit DA, Balcer MD, Phipps GL, West CW, Hoke RA, Ankley GT. **In situ bioassay chamber for assessment of sediment toxicity and bioaccumulation using benthic invertebrates.** Environ Toxicol Chem 1999;18(10):2325-36.
BIOSIS COPYRIGHT: BIOL ABS. In this study, we describe the construction of a simple, inexpensive

bioassay chamber for testing sediment toxicity (survival and growth) and bioaccumulation under field conditions using the midge Chironomus tentans and the oligochaete Lumbriculus variegatus. The test chamber is comprised of a Lexan or Plexiglass core tube containing several screened ports to facilitate water exchange. A rubber stopper, equipped with a small plastic holding vessel to hold organisms, is secured on top of the te nt. Performance in the field test was compared to parallel 10-d laboratory tests. Survival ofC. tentans was 68 and 72% at the two reference sites. Corresponding survival in these sediments in laboratory tests was 96 and 75%. Survival in the transplanted control sediment was 97%. Although significant differences between sediments in the absolute values of survival and growth were observed in both field and laboratory exposures to contaminated sediments, the relative pattern of response for these es and survival of C. tentans. In tests with L. variegatus, survival of worms was 85% in the reference sediment and 40 to 76% in two contaminated sediments. At all sites, a sufficient tissue mass of worms was collected after 10 d to facilitate assessment of bioaccumulation. The results of this study demonstrate that the proposed in situ bioassay can be used successfully to assess toxicity and bioaccumulation in contaminated sediments.

Skinner L, De Peyster A, Schiff K. **Developmental effects of urban storm water in Medaka (Oryzias latipes) and inland silverside (Menidia beryllina).** Arch Environ Contam Toxicol 1999;37(2):227-35.
Stormwater runoff in a coastal urban area (San Diego County, CA) produced significant toxicity to early life stages of medaka (Oryzias latipes) and Menidia (M. beryllina). Exposure of embryos to lower concentrations (5 to 25%) increased the incidence of abnormal swim bladder inflation and other teratogenic responses, whereas higher concentrations resulted in mortality or failure to hatch. Comparisons of EC50s for mortality and failure to hatch with concentrations of individual chemical pollutants (including Cd, Cr, Cu, Pb, Ni, and Zn) revealed low correlations; however, the correlation with total metals was high (-0.84) and corresponded with sample exceedences of Water Quality Criteria (WQC) for Cd, Cu, Pb, and Zn. This strong association between developmental toxicity and toxic metal content of storm water compared favorably with developmental anomalies reported in other studies. Analytical chemistry data for pesticides that may have been in these samples were limited to selected pesticides found usually below detection limits. Greater toxicity of the watershed effluents sampled was generally associated with more developed land surface and less open space. Both medaka and Menidia were found to be useful for studying effects of stormwater on embryonic and early larval development. http://link.springer-ny. com/link/service/journals/00244/bibs/37n2p227.html.

Sunahara GI, Dodard S, Sarrazin M, Paquet L, Hawari J, Greer CW, Ampleman G, Thiboutot S, Renoux AY. **Ecotoxicological characterization of energetic substances using a soil extraction procedure.** Ecotoxicol Environ Saf 1999;43(2):138-48.
The acetonitrile-sonication extraction method (US EPA SW-846 Method 8330) and aquatic-based toxicity tests were used on laboratory and field samples, to characterize the ecotoxicity of soils contaminated with energetic substances. Spiked soil studies indicated that 2,4, 6-trinitrotoluene (TNT)-dependent soil toxicity could be measured in organic extracts and aqueous leachates using the 15-min Microtox (Vibrio fischeri, IC50=0.27 to 0.94 mg TNT/liter incubation medium) and 96-h Selenastrum capricornutum growth inhibition (IC50=0.62 to 1. 14 mg/liter) toxicity tests. Analyses of leachates of composite soil samples [containing TNT and some TNT metabolites, 1,3,5-trinitro-1,3, 5-triazacyclohexane (RDX), and 1,3,5,7-tetranitro-1,3,5, 7-tetrazacyclooctane (HMX)] from an explosives

manufacturing facility, indicated toxicities similar to those found in the TNT-spiked soil studies and pure TNT in solution, and suggested that TNT was the major toxicant. Using TNT as a model toxicant in soils having different moisture contents (20% vs dry) and textures (sandy vs clayey-sandy) but similar organic matter content (3-4%), multi-factorial analyses of Microtox test data revealed that these soil factors significantly influenced the TNT extractability from soil and subsequent toxicity measurements. Taken together, data indicate that the modified Method 8330 may be used in conjunction with ecotoxicity tests to reflect the toxic potential of soils contaminated with energetic substances. Copyright 1999 Academic Press.

Tchounwou PB, Reed L. **Assessment of lead toxicity to the marine bacterium, Vibrio fischeri, and to a heterogeneous population of microorganisms derived from the Pearl River in Jackson, Mississippi, USA.** Rev Environ Health 1999;14(2):51-61.
Microorganisms are known to be excellent test organisms because of the relative ease for handling and suitability for analysis related to their small size, large number and convenient growing conditions. In this research, we tested the toxic effects of lead against a marine bacterium (Vibrio fischeri), and a heterogeneous population of bacteria derived from the Pearl River in Jackson, Mississippi. Using the level of bioluminescence in the Microtox Assay (V. fischeri), and the kinetics of dissolved oxygen uptake and growth (mixed bacterial population) as measures of toxicity, lead concentrations effecting a 50% reduction in these parameters (EC50) were determined as the toxic end-points. The activity quotients were also computed to determine the degrees of toxicity. Optical density (measure of growth) and oxygen uptake were measured over an extended period of time (20 h). EC50 values of 0.34 +/- 0.03, 3.10 +/- 0.01, and 3.80 +/- 0.02 mg/L were recorded for bioluminescence, growth, and oxygen uptake, respectively. As expected, the results indicated that the sensitivity to lead toxicity of V. fischeri was about one order of magnitude (10 times) greater than that of the mixed population of Pearl River microorganisms. Reductions in bioluminescence, growth, and oxygen uptake were directly correlated to lead concentrations, with toxic levels ranging from slightly toxic in lower concentrations to extremely toxic in higher concentrations. Upon 20 h of exposure, the times required to produce 50% reduction in dissolved oxygen uptake were (TD50S) 8.01 +/- 0.44, 9.60 +/- 0.46, 11.29 +/- 0.46, 13.03 +/- 0.57, 17.32 +/- 0.95, and 20.00 +/- 0.00 h in 0, 1, 2, 3, 4, 5, and 6 mg/L of lead, respectively, indicating a time-response relationship with respect to lead toxicity.

Ushirogawa H, Okino T, Hatsushika R. **[Sensitivity of Anisakis larvae to alcoholic and cooling beverages in vitro]**. Kawasaki Igakkai Shi 1998;24(1):47-52. (Jpn)
BIOSIS COPYRIGHT: BIOL ABS. The present experiments were designed to study the sensitivity of Anisakis larvae removed from fresh mackerel to several kinds of alcoholic and cooling beverages in vitro at 37~C. All of the larvae used in the bioassay were identified as Anisakis I-type. The survival period of the larvae in typical solutions was about 5.6 hr in the drinks containing 14 - 25% alcohol, about five days (114 hr) in those containing 2.5 - 10% alcohol and about nine days (225 hr) in the non-alcoholic cooling beverages minutes. This result suggests the possibility that Japanese apricot has a lethal effect on Anisakis larvae.

Vecchi M, Reynoldson TB, Pasteris A, Bonomi G. **Toxicity of copper-spiked sediments to Tubifex tubifex (Oligochaeta, Tubificidae): comparison of the 28-day reproductive bioassay with an early-life-stage bioassay.** Environ Toxicol Chem 1999;18(6):1173-9.

Villeneuve DL, Richter CA, Blankenship AL, Giesy JP. **Rainbow trout cell bioassay-derived relative potencies for halogenated aromatic hydrocarbons: comparison and sensitivity analysis.** Environ Toxicol Chem 1999;18(5):879-88.
BIOSIS COPYRIGHT: BIOL ABS. Rainbow trout hepatoma cells, stably transfected with a luciferase reporter gene under control of dioxin-responsive elements (RLT 2.0 cells) were used to derive relative potencies (RPs) for a variety of halogenated aromatic hydrocarbons (HAHs) that are structurally similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This in vitro bioassay utilizes 96-well microplates, which provide high sample throughput and assay efficiency without affecting sensitivity. The RLT 2.0-derived potencies for dio ecific RPs and RPs based on mammalian bioassays. Sensitivity analysis suggested that the range of uncertainty associated with TCDD equivalent (TEQ) estimates based on RLT 2.0-derived RPs is approximately 10-fold. Within this degree of uncertainty and the context of this study, the RLT 2.0 bioassay showed no definitive biases or inaccuracies relative to similar mammalian- or fish-specific in vitro bioassays. Thus, the RLT 2.0 bioassay appears to be a useful tool for evaluating dioxinlike potency.

GENOTOXICITY AND MUTAGENESIS

Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM, Sarrif AM, Stringer DA, Taalman RD. **Aneuploidy: a report of an ECETOC task force [see comments]**. Mutat Res 1998 Feb;410(1):3-79.
Aneuploidy plays a significant role in adverse human health conditions including birth defects, pregnancy wastage and cancer. Although there is clear evidence of chemically induced aneuploidy in experimental systems, to date there are insufficient data to determine with certainty if chemically induced aneuploidy contributes to human disease. However, since there is no reason to assume that chemically induced aneuploidy will not occur in human beings, it is prudent to address the aneugenic potential of chemicals in the safety assessment process. A wide range of methods has been described for the detection of chemically induced aneuploidy including subcellular systems, tests with fungi, plants and Drosophila as well as in vitro mammalian systems and in vivo mammalian somatic and germ cell assays. However, none of these methods is sufficiently validated or widely used in routine screening. Underlying the efforts to develop aneuploidy-specific assays is the presumption that current genetic toxicology tests do not detected chemicals that have aneuploidy-inducing potential. To address this, we have critically evaluated data from standard genetic toxicology assays for 16 known or suspected aneugens. The conclusions from the review are listed below. 1. At present there are only nine chemicals that can be classified as definitive aneugens, as determined by positive results in in vivo rodent assays. 2. As expected, the majority of definitive and suspected aneugens are negative in the bacterial mutation assay. 3. The majority of definitive aneugens evaluated induce polyploidy in vitro. With few exception, they also induced structural chromosome aberrations in vitro. 4. All of the definitive aneugens that have been sufficiently tested induce micronuclei in rodent bone marrow cells in vivo. A number of these chemicals also induced structural chromosome aberrations in vivo. 5. There is no evidence for a unique germ cell aneugen, that is a chemical that induces aneuploidy in germ cells and not in somatic cells. Furthermore, an analysis of several databases indicates the proportion of chemicals which induce

polyploidy and not chromosome aberrations in vitro is low. Based on these conclusions, the following recommendations are made: for screening purposes, a standard genotoxicity test battery (including an in vitro cytogenetic assay with an assessment of polyploidy and clastogenicity at the same harvest time) should be performed; in the absence of polyploidy induction in vitro no further evaluation of aneuploidy-inducing potential is needed; if polyploidy is observed, in vitro follow-up testing to investigate further the aneuploidy-inducing potential should be conducted; such follow-up testing will generally start with the conduct of a standard in vivo somatic cell micronucleus assay; if the in vivo somatic cell micronucleus assay is negative, with adequate evidence of exposure of the bone marrow to the test compound, no further testing of aneuploidy-inducing potential is needed; if the in vivo somatic cell micronucleus assay is positive, further information on mechanisms of micronucleus induction can be obtained by using kinetochore/centromeric staining in vitro and/or in vivo; an assessment of potential germ cell aneuploidy activity may then be considered; aneuploidy induction which does not involve the direct interaction of a chemical or its metabolite(s) with DNA is expected to have a threshold. This must be considered in the risk assessment of such chemicals; this is not addressed by current risk assessment guidelines.

Altamirano-Lozano M, Valverde M, Alvarez-Barrera L, Molina B, Rojas E. **Genotoxic studies of vanadium pentoxide (V(2)O(5)) in male mice. II. Effects in several mouse tissues.** Teratog Carcinog Mutagen 1999;19(4):243-55.
Vanadium pentoxide (V2O5) was tested for its ability to induce genotoxic damage in six different organs (liver, kidney, lung, spleen, heart, and bone marrow) of mice by using the alkaline Single Cell Gel Electrophoresis (SCGE) assay. Animals were sacrificed 24 h after i.p. administration of the vanadium pentoxide of 23.0, 11.5, or 5.75 microg/g (corresponding to the LD50, 1/2 LD50 and 1/4 LD50, respectively). In all tissues and organs evaluated (except for bone marrow), V2O5 increased the number of cells with damage. Our results showed that i.p. injection of V2O5 induced DNA damage in different organs and tissues, and that this kind of damage can be observed even 24 h after treatment. The analysis of DNA migration and the distribution of DNA damage showed that there are differences in sensitivity between organs and tissues to this compound. In addition the sensitivity of SCGE assay allows the detection of long term DNA damage and the possibility to compare it in various tissues and target organs. Copyright 1999 Wiley-Liss, Inc.

Althaus FR, Kleczkowska HE, Malanga M, Muntener CR, Pleschke JM, Ebner M, Auer B. **Poly ADP-ribosylation: a DNA break signal mechanism.** Mol Cell Biochem 1999;193(1-2):5-11.
Recent evidence obtained with transgenic knockout mice suggests that the enzyme poly(ADP-ribose) polymerase (PARP) does not play a direct role in DNA break processing. Nevertheless, inactivation of the catalytic or the DNA nick-binding functions of PARP affects cellular responses to genotoxins at the level of cell survival, sister chromatid exchanges and apoptosis. In the present report, we conceptualize the idea that PARP is part of a DNA break signal mechanism. In vitro screening studies revealed the existence of a protein family containing a polymer-binding motif of about 22 amino acids. This motif is present in p53 protein as well as in MARCKS, a protein involved in the regulation of the actin cytoskeleton. Biochemical analyses showed that these sequences are directly targeted by PARP-associated polymers in vitro, and this alters several molecular functions of p53- and MARCKS protein. PARP-deficient knockout mice from transgenic mice were found to exhibit several phenotypic features

compatible with altered DNA damage signaling, such as downregulation and lack of responsiveness of p53 protein to genotoxins, and morphological changes compatible with MARCKS-related cytoskeletal dysfunction. The knockout phenotype could be rescued by stable expression of the PARP gene. We propose that PARP-associated polymers may recruit signal proteins to sites of DNA breakage and reprogram their functions.

Amrani S, Rizki M, Creus A, Marcos R. **Genotoxic activity of different chromium compounds in larval cells of Drosophila melanogaster, as measured in the wing spot test.** Environ Mol Mutagen 1999;34(1):47-51.
Two chromium(VI) compounds (potassium chromate and potassium dichromate) and one chromium(III) compound, chromium chloride, were evaluated for genotoxic effects in the wing spot test of Drosophila melanogaster following standard procedures. This assay detects both somatic recombination and mutational events. The genotoxic effects were determined from the appearance of wing spots in flies transheterozygous for the third chromosome recessive markers multiple wing hairs (mwh) and flare-3 (flr (3)), as well as in flies heterozygous formwh and the multiply inverted TM3 balancer chromosome. Genetic changes induced in somatic cells of the wing's imaginal discs lead to the formation of mutant clones on the wingblade. Single spots are due to different genotoxic mechanisms: point mutation, deletion, chromosome breakage, and mitotic recombination; while twin spots are produced only by mitotic recombination. From our results it appears that both chromium(VI) compounds clearly increase the incidence of mutant clones by inducing high increases in the frequency of all types of clones recorded. On the contrary, chromium(III) did not increase the frequency of mutant clones. A high proportion of the total spot induction was due to mitotic recombination, confirming previously reported data on the strong recombinogenic activity of chromium(VI) compounds. Copyright 1999 Wiley-Liss, Inc.

Anderson D, Hambly RJ, Yu TW, Thomasoni F, Shuker DE. **The effect of potassium diazoacetate on human peripheral lymphocytes, human adenocarcinoma Colon caco-2 cells, and rat primary colon cells in the comet assay.** Teratog Carcinog Mutagen 1999;19(2):137-46.
In previous studies, N-(N'-acetyl-L-propyl)-N-nitrosoglycine (APNG) has been shown to be a potent mutagen in a variety of genotoxicity assays and a carcinogen in a limited cancer study. APNG decomposes to a carboxymethyldiazonium ion, which can also be generated from potassium diazoacetate (KDA). KDA is particularly interesting because it is a stable nitrosated derivative of glycine, one of the most common dietary amino acids. KDA has been shown to produce more O6 carboxymethyl- and O6 methyl-adducts than APNG, so it was anticipated that it might also be a potent genotoxic agent. Thus in the present study KDA has been investigated in the single cell gel electrophoresis (Comet) assay, which primarily measures DNA strand breakage. Since KDA has been shown to be formed in the gut, the genotoxic effects of KDA were investigated in vitro in human adenocarcinoma colon Caco-2 cells, and in rat primary colon cells and compared to responses from human peripheral lymphocytes. KDA induced DNA damage in the three cell types, confirming that KDA is genotoxic in a range of mammalian cells.

Anderson D, Yu TW, Hambly RJ, Mendy M, Wild CP. **Aflatoxin exposure and DNA damage in the comet assay in individuals from the Gambia, West Africa.** Teratog Carcinog Mutagen 1999;19 (2):147-55.

The single cell gel electrophoresis assay (Comet assay) was used to measure DNA damage in peripheral lymphocytes from a group of individuals from The Gambia in order to determine whether such damage could be associated with increased exposure to aflatoxin in this population. Responses obtained were correlated to responses previously obtained [1] in a cross-sectional study in the same individuals of various cytogenetic alterations [chromosomal aberrations, micronuclei (crest positive and negative staining), and sister chromatid exchanges], and aflatoxin-albumin adducts. Analysis of variance methods were used to assess the effects of smoking, GSTM1 genotype, sex, age, and smoking status. A comparison was also made between The Gambian individuals and a group of healthy, non-smoking volunteers in the United Kingdom where aflatoxin exposure would be expected to be low. From the earlier study [1], it was determined that the levels of the sister chromatid exchanges and micronuclei were higher in The Gambian group than in a European group where aflatoxin exposure was lower, but that there were no correlations between the adduct levels and the cytogenetic abnormalities at the individual level. In the present study, DNA damage as measured in the Comet assay was not significantly higher than in the healthy United Kingdom volunteers. In addition, there were no associations between cytogenetic damage, GSTM1 genotype, age, sex, lifestyle factors (smoking and aflatoxin exposure), and Comet response at the individual level. Comet response was higher in females than males in The Gambia if one outlier was excluded from analysis and not taking into account other sources of variability. It would appear that DNA damage as measured in the Comet assay in peripheral blood lymphocytes is not a sensitive genotoxic marker of aflatoxin exposure in this population.

Andreoli C, Leopardi P, Rossi S, Crebelli R. **Processing of DNA damage induced by hydrogen peroxide and methyl methanesulfonate in human lymphocytes: analysis by alkaline single cell gel electrophoresis and cytogenetic methods.** Mutagenesis 1999;14(5):497-503.
BIOSIS COPYRIGHT: BIOL ABS. The persistence of induced DNA damage in human lymphocytes after mitogen stimulation and its relationship to subsequent cytogenetic alterations were investigated. The analysis of single-strand breaks and alkali-labile sites by single cell gel electrophoresis (SCGE) showed the almost complete repair of damage induced in resting lymphocytes by methyl methanesulfonate (MMS, 140-210 muM) and hydrogen peroxide (H2O2, 25-100 muM) during the first 16 h of culture. On the other hand, DNA damage was show g the first 16 h of culture, micronuclei were significantly increased at all doses. Conversely, sister chromatid exchange (SCE) rates were increased by chemical treatments to a higher extent in cultures without Ara-C. Delayed treatments, 16 h after mitogen stimulation, led to a significant induction of micronuclei in the case of MMS but not with H2O2. These results suggest that only a minor fraction of DNA damage induced in resting lymphocytes is available for fixation through misreplication, be.

Angelis KJ, Dusinska M, Collins AR. **Single cell gel electrophoresis: detection of DNA damage at different levels of sensitivity.** Electrophoresis 1999;20(10):2133-8.
Single cell gel electrophoresis, also known as the comet assay, is widely used for the detection and measurement of DNA strand breaks. With the addition of a step in which DNA is incubated with specific endonucleases recognising damaged bases, these lesions can be measured, too. In the standard protocol, electrophoresis is carried out at high pH. If, instead, electrophoresis is in neutral buffer, the effect of DNA damage seems to be much reduced--either because alkaline conditions are needed to reveal certain lesions, or because the effect of the same number of breaks on DNA migration is greater at

high pH. A lower sensitivity can be useful in some circumstances, as it extends the range of DNA damage levels over which the assay can be used. Here we compare the performance of standard and modified techniques with a variety of DNA-damaging agents and offer possible explanations for the differences in behaviour of DNA under alternative electrophoretic conditions.

Assad M, Lemieux N, Rivard CH, Yahia LH. **Comparative in vitro biocompatibility of nickel-titanium, pure nickel, pure titanium, and stainless steel: genotoxicity and atomic absorption evaluation.** omed Mater Eng 1999;9(1):1-12.
The genotoxicity level of nickel-titanium (NiTi) was compared to that of its pure constituents, pure nickel (Ni) and pure titanium (Ti) powders, and also to 316L stainless steel (316L SS) as clinical reference material. In order to do so, a dynamic in vitro semiphysiological extraction was performed with all metals using agitation and ISO requirements. Peripheral blood lymphocytes were then cultured in the presence of all material extracts, and their comparative genotoxicity levels were assessed using electron microscopy-in situ end-labeling (EM-ISEL) coupled to immunogold staining. Cellular chromatin exposition to pure Ni and 316L SS demonstrated a significantly stronger gold binding than exposition to NiTi, pure Ti, or the untreated control. In parallel, graphite furnace atomic absorption spectrophotometry (AAS) was also performed on all extraction media. The release of Ni atoms took the following decreasing distribution for the different resulting semiphysiological solutions: pure Ni, 316L SS, NiTi, Ti, and controls. Ti elements were detected after elution of pure titanium only. Both pure titanium and nickel-titanium specimens obtained a relative in vitro biocompatibility. Therefore, this quantitative in vitro study provides optimistic results for the eventual use of nickel-titanium alloys as surgical implant materials.

Au WW, Wilkinson GS, Tyring SK, Legator MS, El Zein R, Hallberg L, Heo MY. **Monitoring populations for DNA repair deficiency and for cancer susceptibility.** Environ Health Perspect 1996 May;104 Suppl 3:579-84.
The induction of a mutator phenotype has been hypothesized to cause the accumulation of multiple mutations in the development of cancer. Recent evidence suggests that the mutator phenotype is associated with DNA repair deficiencies. We have been using a challenge assay to study exposed populations to test our hypothesis that exposure to environmental toxicants induce DNA repair deficiency in somatic cells. In this assay, lymphocytes were irradiated in vitro to challenge cells to repair the radiation-induction DNA strand breaks. An increase of chromosome aberrations in the challenged cells from toxicant-exposed populations compared to nonexposed populations is used to indicate abnormal DNA repair response. From studies of cigarette smokers, butadiene-exposed workers, and uranium-exposed residents, the assay showed that these exposed populations had mutagen-induced abnormal DNA repair response. The phenomenon was also demonstrated using experimental animals. Mice were exposed in vivo to two different doses of N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) and their lymphocytes were challenged with one dose of a radiomimetic chemical, bleomycin, in vitro. These challenged lymphocytes showed an MNNG dose-dependent increase of abnormal DNA repair response. In a population that was potentially exposed to teratogens--mothers having children with neural tube defects--lymphocytes from these mothers did not have the abnormal response in our assay. In studies with patients, we reported that lymphocytes from Down's syndrome patients have the abnormal DNA repair response. Lymphocytes from skin cancer-prone patients (epidermodysplasia

verruciformis) have normal response to gamma-ray challenge but abnormal response to UV-light challenge. These patient studies also indicate that the challenge assay is useful in documenting the radiosensitivity of Down's syndrome and the UV sensitivity in EV patients. In most cases, the challenge assay is more sensitive in detecting biological effects than the standard chromosome aberration assay. Our series of studies indicates that the challenge assay can be used to document biological effects from exposure to mutagens and that the effect is an abnormal DNA repair response. This abnormality can increase the risk for development of cancer. The repair deficiency is currently being validated using a plasmid transfection (host-reactivation) assay. The need to integrate chromosome aberration and the challenge assays with other relevant assays for better documentation of biological effects and for more precise prediction of health risk will be presented. Our experience in using genetic polymorphism and host-reactivation assays will be discussed.

Barrera Ferrer SM, Villalobos Cabrera HD. **[Genotoxic effects of vanadyl sulfate in Drosophila melanogaster]**. Invest Clin 1998 Apr;39 Suppl 1:123-37. (Spa)
This study presents the analysis of chemically induced somatic mutation in Drosophila larvae assayed later as single light (LS) mosaic spots in the adult eye. The larvae were treated with Vanadyl sulphate (VOSO4), the highest exposure was 10.0, 8.0, 6.0 and 4.0 mM which was the acutely lethal concentration, while than lowest exposure of 2.5, 2.0 and 1.0 mM, cause reproductive effects and genotoxic activity, compared to the control. Previous to the realization of the mutagenicity assay was determined the lethal toxicity of the compound undertest since the concentration-mortality relationship is a useful indicator of the biological activity of Vanadium. The toxic effect in fly adult was to compare any differences in the sensitivity of males (white) and female (oregon), observing the higher sensitivity of the males. The date presented with tests SMART and their statistical evaluation lead to the following conclusions. To test the two hypotheses was propose to apply the conditional binomial test (Kastenbaum and Bowman, 1970) or the X2 test for proportions (K. Pearson criterion). Each hypothesis was tested at the 5% significance level. In conclusion the Vanadyl Sulphate produce aberrant red sector in w+/w, it is not correlated with the capacity of an agent to induce chromosomal damage and mitotic recombination, but rather seems positively correlated with its weak capacity to produce points mutations.

Bauch T, Bocker W, Mallek U, Muller WU, Streffer C. **Optimization and standardization of the "comet assay" for analyzing the repair of DNA damage in cells.** Strahlenther Onkol 1999;175(7):333-40.
BACKGROUND: The "comet assay" has become an interesting and a very useful tool for the analysis of the induction and amount of DNA damage in single cells thus offering the opportunity to measure the effectiveness of DNA repair. On the basis of the Ostling and Johanson protocol we have developed a modified method with increased sensitivity and high reproducibility. MATERIAL AND METHODS: Human tumor cells or isolated human peripheral blood lymphocytes were analyzed in the experiments. The amount of DNA damage and the effectiveness of DNA repair was measured after X-irradiation using the "comet assay" technique. RESULTS: In this presentation the influences of different methodological factors like agarose concentration, buffer pH, electrophoresis time, electric field strength on the applicability of the "comet assay" are described in detail and optimum conditions for "comet assay" experiments have been evaluated. Additionally the authors will show a comparison of different fluorescent DNA dyes pointing out their advantages or disadvantages for "comet" analysis. The

usefulness of this technique and its capabilities are exemplified by showing DNA repair kinetics of human lymphocytes of different healthy or radiosensitive donors after in-vitro irradiation with 2 Gy X-rays. CONCLUSIONS: This paper presents data on the optimization and standardization of the original "comet assay" leading to an extremely fast and practicable protocol in the field of single cell gel electrophoresis. After irradiation with 0.1 Gy an increase in the amount of DNA damage can be measured with high statistical significance and the DNA repair capacity of individual cells after X-ray doses of 2 Gy can be analyzed with high reproducibility. The results comparing DNA repair capacities of different donors point out that the "comet assay" may have the potential for the estimation of individual radiosensitivity.

Bebenek A, Smith LA, Drake JW. **Bacteriophage T4 rnh (RNase H) null mutations: effects on spontaneous mutation and epistatic interaction with rII mutations.** J Bacteriol 1999;181(10):3123-8.
The bacteriophage T4 rnh gene encodes T4 RNase H, a relative of a family of flap endonucleases. T4 rnh null mutations reduce burst sizes, increase sensitivity to DNA damage, and increase the frequency of acriflavin resistance (Acr) mutations. Because mutations in the related Saccharomyces cerevisiae RAD27 gene display a remarkable duplication mutator phenotype, we further explored the impact of rnh mutations upon the mutation process. We observed that most Acr mutants in an rnh+ strain contain ac mutations, whereas only roughly half of the Acr mutants detected in an rnhDelta strain bear ac mutations. In contrast to the mutational specificity displayed by most mutators, the DNA alterations of ac mutations arising in rnhDelta and rnh+ backgrounds are indistinguishable. Thus, the increase in Acr mutants in an rnhDelta background is probably not due to a mutator effect. This conclusion is supported by the lack of increase in the frequency of rI mutations in an rnhDelta background. In a screen that detects mutations at both the rI locus and the much larger rII locus, the r frequency was severalfold lower in an rnhDelta background. This decrease was due to the phenotype of rnh rII double mutants, which display an r+ plaque morphology but retain the characteristic inability of rII mutants to grow on lambda lysogens. Finally, we summarize those aspects of T4 forward-mutation systems which are relevant to optimal choices for investigating quantitative and qualitative aspects of the mutation process.

Becerril C, Ferrero M, Sanz F, Castano A. **Detection of mitomycin C-induced genetic damage in fish cells by use of RAPD.** Mutagenesis 1999;14(5):449-56.
BIOSIS COPYRIGHT: BIOL ABS. Concern about genetic alterations in fish populations arising from anthropogenic activities has led to the adaptation and/or development of new tests and techniques that shed light on these alterations. The high number and the reduced size of chromosomes and the long cell cycle associated with most fish species preclude the use of most accepted genotoxicity assays. The purpose of this work was to study the capability of the randomly amplified polymorphic DNA technique to show genotoxic effects i arison between the control and exposed fingerprints for 4, 6 and 8 h. Results show that 5 ng of DNAtemplate and 4 pM chosen primer were optimum to show differences between control and exposed cells and to obtain reproducible results. The results obtained, after optimum conditions were established, show that this system could be useful for the assessment of DNA alterations in in vitro genotoxicity studies.

Belyaev IY, Eriksson S, Nygren J, Torudd J, Harms-Ringdahl M. **Effects of ethidium bromide on DNA loop organisation in human lymphocytes measured by anomalous viscosity time dependence and single cell gel electrophoresis.** Biochim Biophys Acta 1999;1428(2-3):348-56.

The effects of ethidium bromide (EtBr) on human lymphocytes were studied by the method of anomalous viscosity time dependence (AVTD) and by the comet assay. EtBr at low concentrations increased the maximum viscosity and time of radial migration as measured with AVTD at neutral conditions of lysis. A pronounced relaxation of DNA loops was observed with the neutral comet assay. The maximal comet length corresponded to 2 Mb DNA loops. At high concentrations of EtBr, 2 mg/ml, significant reduction in AVTD below control level was seen that suggested hypercondensation of chromatin. The hypercondensation was directly observed with the neutral comet assay. EtBr did not induce DNA strand breaks as measured by the alkaline comet assay. The hypercondensed nuclei could be decondensed by irradiation with gamma-rays or exposure to light. The data provide evidence that EtBr at high concentrations resulted in hypercondensation of chromatin below control level. The comet assay confirmed that the increase in AVTD peaks deals with relaxation of loops and AVTD decrease is caused by chromatin condensation. The prediction of the AVTD theory for a correlation between time of radial migration and condensation of chromatin was verified. Further, the data show that the comet assay at neutral conditions of lysis is rather sensitive to DNA loop relaxation in the absence of DNA damage. Finally, donor specificity was found for the hypercondensation.

Bermudez E, Ferng SF, Castro CE, Mustafa MG. **DNA strand breaks caused by exposure to ozone and nitrogen dioxide.** Environ Res 1999;81(1):72-80.
The present study demonstrates that exposure to ozone (O3) and nitrogen dioxide (NO2) can cause DNA single-strand breaks in alveolar macrophages. Three-month-old male Sprague-Dawley rats, specific pathogen free, were exposed to either 1.2 ppm NO2 or 0.3 ppm O3 alone or a combination of these two oxidants continuously for 3 days. The control group was exposed to filtered room air. The oxidant effects were substantiated by determining total and differential cell counts, lactate dehydrogenase activity, and total soluble protein in bronchoalveolar lavage. DNA damage was measured as single-strand breaks by alkaline elution assay. The results showed that, relative to control, NO2 exposure did not cause any significant change in the parameters studied. Exposure to O3 and combined exposure to NO2 and O3 caused significant changes in all parameters studied except cell viability. The rates of elution (Kc) of single-strand DNA from polycarbonate filter for O3 exposure and combined exposure were 73 and 79% faster than that of the control, respectively. The amounts of DNA single-strand breaks caused by O3 and combined exposure were significantly greater than the amounts detected for the NO2-exposed and control groups. Copyright 1999 Academic Press.

Bhaskaran A, May D, Rand-Weaver M, Tyler CR. **Fish p53 as a possible biomarker for genotoxins in the aquatic environment.** Environ Mol Mutagen 1999;33(3):177-84.
The p53 gene is a tumour suppressor gene which has a fundamental role in cell cycle control and division, and in mammals certain genotoxic agents induce specific mutations in p53, leading to tumourigenesis. Fish have been investigated as models for studying carcinogens, but as yet very little data exists that links exposure to specific chemicals with the aetiology of tumours found in wild populations. In this study, p53 was sequenced from five species of fish with a view to the possible use of mutations in the highly conserved domains of p53 to identify genotoxins in the aquatic environment. A 0.8 kb fragment of the cDNA encompassing the conserved DNA-binding domain of p53 was sequenced in three Oncorhynchus salmonid fish: coho (O. kisutch), chum (O. keta), and chinook (O. tshawytscha) and full-length p53 cDNAs were sequenced in the puffer fish (Tetraodon miurus) and the barbel (Barbus

barbus). The full-length puffer fish and barbel p53 cDNAs were 1834 bp and 1790 bp in length, encoding a 367 aa protein and a 369 aa protein, respectively. The deduced aa sequences of the p53 cDNA in the Oncorhynchus salmon shared a 100% identity in the five conserved regions (I-V). Comparisons of the deduced aa sequences for puffer fish and barbel p53 with other fish p53s revealed a high homology within the conserved DNA binding domain (68-86% for puffer fish and between 66-88% for barbel). "Conserved" domain I was not highly conserved in fish, as it is in mammals, and, therefore, conserved domains II-V are most likely to provide the valuable sequences in fish p53 for use in mutational studies to fingerprint genotoxins in the aquatic environment.

Blasiak J, Trzeciak A, Malecka-Panas E, Drzewoski J, Iwanienko T, Szumiel I, Wojewodzka M. **DNA damage and repair in human lymphocytes and gastric mucosa cells exposed to chromium and curcumin.** Teratog Carcinog Mutagen 1999;19(1):19-31.
Human population can be considered as a subject of combined exposure to chemicals. Hexavalent chromium is a well-known mutagen and carcinogen. Curcumin, a popular spice and pigment, is reported to have antineoplastic properties. The single cell gel electrophoresis (Comet assay) is a sensitive technique that allows detecting double- and single-strand DNA breaks caused by a broad spectrum of mutagens. In the present work the ability of curcumin to reduce DNA damage induced by chromium in human lymphocytes and gastric mucosa (GM) cells was investigated by using the comet assay. Chromium at 500 microM evoked DNA damage measured as significant ($P < 0.001$), about a two-fold increase in comet tail moment of both lymphocytes and GM cells. Curcumin at 10, 25, and 50 microM also damaged DNA of both types of cells in a dose-dependent manner: the increase in the tail moment reached about twenty times of the control value ($P < 0.001$). The combined action of chromium at 500 microM and curcumin at 50 microM resulted in the significant ($P < 0.001$) increase in the comet tail moment of both types of cells. In each case, treated cells were able to recover within 60 min. Our study clearly demonstrates that curcumin does not inhibit DNA damaging action of hexavalent chromium in human lymphocytes and GM cells. Moreover, curcumin itself can damage DNA of these cells and the total effect of chromium and curcumin is additive. Further studies are needed to establish the role of interaction of curcumin with DNA in carcinogenesis.

Bolognesi C, Lando C, Forni A, Landini E, Scarpato R, Migliore L, Bonassi S. **Chromosomal damage and ageing: effect on micronuclei frequency in peripheral blood lymphocytes.** Age Ageing 1999;28 (4):393-7.
BACKGROUND: Instability in the organization and expression of the genetic material has been hypothesized as the basic mechanism of ageing. OBJECTIVE: To quantify the effect of ageing on chromosomal damage as measured by spontaneous micronuclei (MN) frequency in peripheral blood lymphocytes. METHOD: Analysis of a large population sample from two laboratories applying the cytokinesis-block technique and a third using traditional interphase analysis. The age-related effect on baseline level of micronuclei frequency and on cell proliferation measures was further investigated in a study of peripheral blood samples from healthy subjects. RESULTS: There was an increase of MN frequency with age. The regression lines showed a positive slope and were statistically significant ($P< 0.01$) with a steeper trend for cytochalasin B-treated samples. An inverse correlation with age was detected for the percentage of binucleated cells in laboratories using cytochalasin B. This study confirms the increase of basal level of MN with age. A decrease by age in proliferation efficiency measured by

the percentage of binucleated cells suggests an interference of age-related factors on cell division. CONCLUSION: There is an increase in MN frequency with increasing age.

Boturyn D, Constant JF, Defrancq E, L'homme J, Barbin A, Wild CP. **A simple and sensitive method for in vitro quantitation of abasic sites in DNA.** Chem Res Toxicol 1999;12(6):476-82.
A novel method for the quantitation of abasic sites (AP sites) in DNA is described. As abasic sites can be generated by controlled thermal treatment of base-modified DNA, this method can be used for estimation of the extent of DNA damage resulting from exposure to genotoxic agents. The method involves use of probe molecules 1 and 2 that contain a fluorescent label linked to an aminooxy group which reacts specifically with the aldehydic function of the ring-opened form of abasic sites. The two fluorescent probes 1 and 2 were found to react with 2-deoxyribose, a model substrate, at the optimum of pH 4.0. As spontaneous depurination occurs at low pH, the reactions with abasic DNA were carried out at neutral pH with an excess concentration of the probes. Studies with alkylated, depurinated calf thymus DNA showed that the method is selective and quantitative. Good correlations were found between the level of 7-methylguanine (7-MeGua), generated in vitro in DNA by the methylating agent dimethyl sulfate, and the amount of AP sites as determined by the method presented here. In addition, similar correlations were found when the assay was used to detect abasic sites in DNA isolated from rats treated with carcinogenic alkylating agents. In each case, the level of abasic sites, as expected, is slightly higher than the level of 7-MeGua which is known to represent about 70% of the total modifications of DNA following exposure to the methylating agent. This method may be useful not only in experimental settings but also in studies of DNA damage in humans resulting from chemotherapy or exposure to environmental agents.

Bourachot B, Yaniv M, Muchardt C. **The activity of mammalian brm/SNF2alpha is dependent on a high-mobility-group protein I/Y-like DNA binding domain.** Mol Cell Biol 1999;19(6):3931-9.
The mammalian SWI-SNF complex is a chromatin-remodelling machinery involved in the modulation of gene expression. Its activity relies on two closely related ATPases known as brm/SNF2alpha and BRG-1/SNF2beta. These two proteins can cooperate with nuclear receptors for transcriptional activation. In addition, they are involved in the control of cell proliferation, most probably by facilitating p105(Rb) repression of E2F transcriptional activity. In the present study, we have examined the ability of various brm/SNF2alpha deletion mutants to reverse the transformed phenotype of ras-transformed fibroblasts. Deletions within the p105(Rb) LXCXE binding motif or the conserved bromodomain had only a moderate effect. On the other hand, a 49-amino-acid segment, rich in lysines and arginines and located immediately downstream of the p105(Rb) interaction domain, appeared to be essential in this assay. This region was also required for cooperation of brm/SNF2alpha with the glucocorticoid receptor in transfection experiments, but only in the context of a reporter construct integrated in the cellular genome. The region has homology to the AT hooks present in high-mobility-group protein I/Y DNA binding domains and is required for the tethering of brm/SNF2alpha to chromatin.

Cai Y, Zhuang Z. **[DNA damage in human peripheral blood lymphocyte caused by nickel and cadmium]**. Zhonghua Yufang Yixue Zazhi 1999;33(2):75-7. (Chi)
BIOSIS COPYRIGHT: BIOL ABS. Objective To understand the different implication of various forms of DNA damage in genotoxicity of nickel and cadmium. Methods Human peripheral lymphocyte was exposed to nickel chloride and cadmium chloride in vitro. Levels of DNA single-and double-strand

breaks and DNA-protein crosslinks in human peripheral lymphocyte were determined with single cell gel electrophoresis (SCGE). Activity of poly (ADP-ribose) polymerase (PARP) was determined by (3H)-NAD incorporating method. Results Levels of DN dmium) could induce the cleavage of DNA and activate PARP, and high levels of the two kinds of metal (2.00 - 10.00 mumol/L of nickel and 0.80 - 20.00 mumol/L of cadmium) could not induce the enzyme cleavage of DNA. Conclusion Formation and cleavage of DNA double strand and blockage of activation of PARP can play an important role in carcinogenesis and mutagenesis.

Charles JM, Cunny HC, Wilson RD, Bus JS, Lawlor TE, Cifone MA, Fellows M, Gollapudi B. **Ames assays and unscheduled DNA synthesis assays on 2,4-dichlorophenoxyacetic acid and its derivatives.** Mutat Res 1999;444(1):207-16.
BIOSIS COPYRIGHT: BIOL ABS. 2,4-Dichlorophenoxyacetic acid and several of its derivatives (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The genetic toxicity in vitro of 2,4-D and seven of its salts and esters were examined by employing gene mutation in bacteria (Ames test) and induction of DNA damage and repair in rat hepatocytes. In addition, an in vivo unscheduled DNA synthesis (UDS) assay was performed on 2,4-D. There were no indications of genotoxic potential.

Charles JM, Cunny HC, Wilson RD, Ivett JL, Murli H, Bus JS, Gollapudi B. **In vivo micronucleus assays on 2,4-dichlorophenoxyacetic acid and its derivatives.** Mutat Res 1999;444(1):227-34.
The potential for 2,4-D and seven of its salts and esters to induce cytogenetic abnormalities in mammalian cells in vivo was investigated in the mouse bone marrow micronucleus test. All the test materials were administered to male and female mice by oral gavage and the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow were determined at intervals of 24, 48 and 72 h following dosing. There were no significant increases in the incidence of MN-PCE in the treated mice at any of the bone marrow sampling times. These results are consistent with the reported lack of in vitro genetic toxicity for these materials in various in vitro genotoxicity assays as well as the absence of carcinogenic potential for 2,4-D in both mice and rats.

Chen G, Dubrawsky I, Mendez P, Georgiou G, Iverson BL. **In vitro scanning saturation mutagenesis of all the specificity determining residues in an antibody binding site.** Protein Eng 1999;12(4):349-56.
For the first time, each specificity determining residue (SDR) in the binding site of an antibody has been replaced with every other possible single amino acid substitution, and the resulting mutants analyzed for binding affinity and specificity. The studies were conducted on a variant of the 26-10 antidigoxin single chain Fv (scFv) using in vitro scanning saturation mutagenesis, a new process that allows the high throughput production and characterization of antibody mutants [Burks,E.A., Chen,G., Georgiou,G. and Iverson,B.L. (1997) Proc. Natl Acad. Sci. USA, 94, 412-417]. Single amino acid mutants of 26-10 scFv were identified that modulated specificity in dramatic fashion. The overall plasticity of the antibody binding site with respect to amino acid replacement was also evaluated, revealing that 86% of all mutants retained measurable binding activity. Finally, by analyzing the physical properties of amino acid substitutions with respect to their effect on hapten binding, conclusions were drawn regarding the functional role played by the wild-type residue at each SDR position. The reported results highlight the value of in vitro scanning saturation mutagenesis for engineering antibody binding specificity, for

evaluating the plasticity of proteins, and for comprehensive structure-function studies and analysis.

Chene P. **Mutations at position 277 modify the DNA-binding specificity of human p53 in vitro.** Biochem Biophys Res Commun 1999;263(1):1-5.
p53 regulates the expression of different genes that contain in their promoter a DNA sequence with two copies of the 10-base motif Pu(1)Pu(2)Pu(3)C(4)(A/T)(5)(T/A)(6)G(7)Py(8)Py(9)Py(10). This sequence is degenerated, and thymine or cytidine is found equally at position 3 or 8. These two bases make contact with cysteine-277 of the human p53. An in vitro study was carried out to determine whether p53 could be mutated at position 277 so that it binds preferentially to a sequence containing thymine or cytidine. Various mutant proteins were created and their DNA-binding specificity was determined by gel shift assay. Two of them show an altered specificity. The Cys277Ser protein binds preferentially to cytidine-containing sequences while the Cys277Ala mutant has a preference for thymine-containing sequences. This specificity is presumably achieved because an alanine residue at position 277 interacts with the thymine via hydrophobic interactions and a serine makes a hydrogen bond with the cytidine but not with the thymine. Copyright 1999 Academic Press.

Courtemanche C, Anderson A. **The p53 tumor suppressor protein reduces point mutation frequency of a shuttle vector modified by the chemical mutagens (:)7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene, aflatoxin B1 and meta-chloroperoxybenzoic acid.** Oncogene 1999;18 (33):4672-80.
BIOSIS COPYRIGHT: BIOL ABS. p53 has been postulated to be the guardian of the genome. However, results supporting the prediction that point mutation frequencies are elevated in p53-deficient cells either have not been forthcoming or have been equivocal. To analyse the effect of p53 on point mutation frequency, we used the supF gene of the pYZ289 shuttle vector as a mutagenic target. pYZ289 was treated in vitro by ultraviolet irradiation, aflatoxin B1, (:)7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene and meta-c t a higher level of premutational lesions for aflatoxin B1 and (:)7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene and at a lower level of lesions for meta-chloroperoxybenzoic acid. This suggests that the chemical mutagens produce, in a dose-dependent fashion, two kinds of DNA damage, one subject to p53-dependent mutation frequency reduction and the other not. These results indicate that p53 can reduce the point mutation frequency in a shuttle vector treated by chemical mutagens and su.

Crebelli R, Carere A, Leopardi P, Conti L, Fassio F, Raiteri F, Barone D, Ciliutti P, Cinelli S, Vericat JA. **Evaluation of 10 aliphatic halogenated hydrocarbons in the mouse bone marrow micronucleus test.** Mutagenesis 1999;14(2):207-15.
Ten halogenated aliphatic hydrocarbons (carbon tetrachloride, 1-chlorohexane, 2,3-dichlorobutane, 1,2-dichloroethane, 1,2-dichloroethylene, 1,3-dichloropropane, hexachloroethane, 1,1,2-trichloroethane, 1,2,3-trichloropropane and 1,1,3-trichloropropene), previously assayed in genetic assays in fungi, were evaluated in the mouse bone marrow micronucleus test in order to assess their genotoxicity in vivo. All chemicals were administered once i.p. at 40 and 70-80% of their respective LD50 to male and female CD-1 mice, 24 and 48 h before killing. All treatments produced evident clinical symptoms, but no marked depression of bone marrow proliferation. No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes over the control values were observed at any sampling time with any of the 10 halogenated hydrocarbons assayed. The comparison of the results obtained in this

study with the findings provided by in vitro micronucleus assays on the same chemicals, reported by other authors, indicate that mouse bone marrow is weakly sensitive to the genotoxic effects induced by halogenated hydrocarbons in other test systems. This suggests that the role of such an assay in carcinogen screening may be questionable for this chemical class. An examination of mouse bone marrow micronucleus test results with the halogenated aliphatic hydrocarbons classified as carcinogens by IARC supports this conclusion.

Cromie KD, Ahmad K, Malik T, Buyukuslu N, Glass RE. **Trans-dominant mutations in the 3'-terminal region of the rpoB gene define highly conserved, essential residues in the beta subunit of RNA polymerase: the GEME motif.** Genes Cells 1999;4(3):145-59.
BACKGROUND: The multimeric DNA-dependent RNA polymerases are widespread throughout nature. The RNA polymerase of Escherichia coli, which is the most well characterized, consists of a holoenzyme with subunit stoichiometry of alpha2betabeta'sigma. The beta subunit is conserved and has been implicated in all stages of transcription. The extreme C-terminus of the beta subunit, which includes two well-conserved sequence segments, contributes to the active centre and has been proposed to act in transcriptional termination. We describe a genetic system for further characterizing the role of the extreme C-terminus of the beta subunit of E. coli RNA polymerase. This involves random, PCR (Polymerase Chain Reaction)-mediated mutagenesis of the 3' region of rpoB encoding the C-terminal 116 amino acids of beta, followed by the isolation and characterization of trans-dominant-negative mutations. RESULTS: Substitutions of conserved residues in this region were obtained that exhibited different degrees of growth inhibition in a host expressing the chromosomal-encoded wild-type form of the beta subunit. A number of different substitutions were isolated within the highly conserved sequence motif GEME (residues 1271-->1274 of the E. coli beta subunit). In addition, substitutions were obtained in the extreme C-terminal (surface-exposed) region of beta and at two residues previously proposed to be in the active site (H1237, K1242). The properties of the purified mutant holoenzymes, assessed by transcription assays in vitro, suggested a promoter blockading action. CONCLUSIONS: We have identified an important, highly conserved motif in the beta subunit, GEME (residues 1271-->1274). The nature and effect of the amino acid substitutions at the Gly residue in GEME emphasize the importance of a small, uncharged residue at this position. The in vitro properties of the most extreme trans dominant-negative mutants altered in the GEME motif (and the mutant characteristics in vivo) were similar to those of certain previously identified active-site mutants, suggesting that the altered RNA polymerases were capable of promoter binding and RNA chain initiation but were deficient in the subsequent transcriptional stage.

Dang LN, McQueen CA. **Mutagenicity of 4-aminobiphenyl and 4-acetylaminobiphenyl in Salmonella typhimurium strains expressing different levels of N-acetyltransferase.** Toxicol Appl Pharmacol 1999;159(2):77-82.
4-Aminobiphenyl (4-ABP), an aromatic amine present in tobacco smoke, is an animal and human carcinogen. 4-ABP can undergo several biotransformation reactions to yield DNA-binding species. The role of acetylation in the biotransformation of 4-ABP to reactive intermediates was investigated by determining mutagenicity in Salmonella typhimurium strains expressing various levels of acetyltransferases (NAT/OAT). Strain YG1029, which has multiple copies of the NAT/OAT gene, was the most sensitive to 4-ABP. With rat S9 activation, 4-ABP (5 micrograms/plate) induced 789 +/- 98

revertants/plate. At that concentration, an average of 200 revertants/plate was seen in both TA100, which has a single copy of the NAT/OAT gene, and in TA100/1,8DNP6, which is NAT/OAT deficient. This pattern was also present when the bacteria were exposed to the acetylated derivative, 4-acetylaminobiphenyl (4-AABP). At 10 micrograms/plate, 4-AABP induced 855 +/- 47 revertants/plate in YG1029 while 169 +/- 39 and 149 +/- 28 revertants/plate were observed in strains TA100 and TA100/1,8DNP6, respectively. The mutagenic profiles of 4-ABP and 4-AABP observed with the mouse S9 activating system were similar to that seen with the rat. These data establish a correlation between increased bacterial NAT/OAT activity and increased mutagenicity of 4-ABP. Results with both 4-ABP and 4-AABP support acetylation of the oxygen to be a key step in activation.

Davies R, Gant TW, Smith LL, Styles JA. **Tamoxifen induces G:C-->T:A mutations in the cII gene in the liver of lambda/lacI transgenic rats but not at 5'-CpG-3' dinucleotide sequences as found in the lacI transgene.** Carcinogenesis 1999;20(7):1351-6.
Tamoxifen, a rat liver carcinogen, can induce mutations in the lacI gene in the livers of lambda/lacI transgenic rats. However, the presence of persistent tamoxifen adducts on the liver DNA raises the possibility that some contribution to the mutagenesis from ex vivo mutations during the in vitro lacI assay cannot be ruled out. To address this issue, mutagenesis at the cII gene of the transgenic shuttle vector was determined using a selection based assay which is unaffected by the presence of tamoxifen-DNA adducts. Female lambda/lacI transgenic rats were dosed orally with tamoxifen (20 mg/kg body wt) daily for 6 weeks, causing a 3.2-fold increase in the mutant frequency (MF) in the cII gene compared with that obtained with solvent treated animals. This was similar to the MF found previously at the lacI gene and confirms that tamoxifen is mutagenic in vivo. The major class of mutation induced by tamoxifen in the cII gene was G:C-->T:A transversions as was found previously in the lacI gene. However, in the one unreplicated study of mutations in the p53 gene of liver tumours induced by tamoxifen, no G:C-->T:A transversions were found; possible differences between mutagenesis in normal and tumour tissues are explored. The major proportion of the G:C-->T:A transversions occurred at 5'-CpG-3' dinucleotide (CpG) sites in the lacI gene, but not at such sites in the cII gene. The methylation of CpG sites greatly enhances the targeting of deoxyguanosine by carcinogens, thus this finding might be explained by differences in the methylation patterns at their respective CpG sites; however, nothing is known about the methylation status of either the lacI nor the cII gene in this transgenic rat. This study raises the important issue of which target genes (mammalian or transgenic) should be used as endpoints in mammalian mutagenesis assays.

De Flora S, Bagnasco M, Vainio H. **Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: mechanistic issues.** Mutagenesis 1999;14(2):153-72.
BIOSIS COPYRIGHT: BIOL ABS. The mechanisms involved in the modulation of genotoxic and related effects by carotenoids and vitamin A were inferred from a critical review of an ad hoc constructed database. Almost 500 results were generated in experimental models evaluating the activity of 32 structurally, metabolically and functionally related nutrients, including beta-carotene and 26 other carotenoids, retinol, retinal, all-trans-retinoic acid and retinyl esters. As many as 67 experimental test systems, either in vitro or i uch as inhibition of N-myc gene expression resulting in antiproliferative effects, up-regulation of cell-to-cell communication, an increase in connexin 43 gene expression, a decrease in the 'spontaneous' cell transformation frequency and induction of differentiation in vitro. A

large number of studies investigated the modulation by carotenoids and vitamin A of genotoxic and related effects produced by 69 genotoxicants, including biological agents, physical agents, chemical compounds and complex which require metabolic activation to electrophilic derivatives in either bacterial or mammalian cells. Coupled with biochemical data, the distinctive patterns observed with genotoxic agents belonging to different chemical classes suggest a complex modulation of both phase I and phase II enzymes involved in the metabolism of xenobiotics. Furthermore, carotenoids and vitamin A shared other protective mechanisms, such as scavenging of genotoxic oxygen species, modulation of signal transduction pat nt with the recognized cancer-preventive activity of these nutrients in certain animal models and with the evidence provided by observational epidemiological studies, which suggested cancer-protective effects at many sites as related to their dietary intake or plasma levels. However, all these lines of evidence and mechanistically based premises contrast with the unexpected outcome of recent clinical intervention trials, which raised the concern that supplemental use of beta-carotene and vitamin.

De Mejia EG, Castano-Tostado E, Loarca-Pina G. **Antimutagenic effects of natural phenolic compounds in beans.** Mutat Res 1999;441(1):1-9.
Polyphenols in fruits, vegetables (e.g., flavonols like quercetin) and tea (e.g., catechins such as epigallocatechin gallate) are good antioxidants with antimutagenic and anticarcinogenic properties. In the present study, the Salmonella typhimurium tester strain YG1024 was used in the plate-incorporation test to examine the antimutagenic effect of phenolic compounds, extracted from common beans (Phaseolus vulgaris), on 1-NP and B[a]P mutagenicity. Dose-response curves for 1-NP and B[a]P were obtained; the number of net revertants/plate at the peak mutagenic dosage were 880 for 1-NP and 490 for B[a]P. For the antimutagenicity studies doses of 0.1 microg/plate and 2 microg/plate for 1-NP and B [a]P, respectively, were chosen. We obtained a dose-response curve of ellagic acid (EA) against B[a]P and 1-NP mutagenicity. To test the bean extract, a dose of 300 microg/plate of EA was chosen as the antimutagenic control. The EA and bean extracts were not toxic to the bacteria at the concentrations tested. The inhibitory effects of the bean extracts and EA against B[a]P mutagenicity were dose-dependent. The percentages of inhibition produced against B[a]P (2 microg/plate) using 300 microg/ plate of EA and for the extracts 500 microg equivalent catechin/plate were 82%, 83%, 81% and 83% for EA, water extract, water/methanol extract and methanol extract, respectively. However, for 1-NP mutagenicity, only the methanolic extract from beans showed an inhibitory effect. These results suggest that common beans, as other legumes, can function as health-promoting foods. Copyright 1999 Elsevier Science B.V.

De Sa Ferreira IC, Ferrao Vargas VM. **Mutagenicity of medicinal plant extracts in Salmonella/ microsome assay.** Phytother Res 1999;13(5):397-400.
Aqueous extracts of medicinal plants used in south Brazilian folk medicine (Myrciaria tenella, Smilax campestris, Tripodanthus acutifolius and Cassia corymbosa) were screened for the presence of mutagenic activity in the Salmonella/microsome assay. Signs of an increased mutagenic induction below revertant rates, which is two times the spontaneous yields, were detected for all extracts. The extracts of C. corymbosa showed signs of mutagenic activity in the following strains: TA100 with and without metabolization and TA98 after metabolization. These mutagenic signs were observed in the M. tenella extracts without metabolization. S. campestris and T. acutifolius infusions also showed signs indicative

of direct mutagenic activity in the TA98 strain. The positive results may be related to the presence of flavonoids, tannins and anthraquinones in the extracts. Copyright 1999 John Wiley & Sons, Ltd.

Delgado-Rodriguez A, Ortiz-Marttelo R, Villalobos-Pietrini R, Gomez-Arroyo S, Graf U. **Genotoxicity of organic extracts of airborne particles in somatic cells of Drosophila melanogaster.** Chemosphere 1999;39(1):33-43.
BIOSIS COPYRIGHT: BIOL ABS. Complex mixtures extracted from air filters exposed for 24 h in two sessions (27 July and 02 August 1991) and at two locations (Merced, downtown, and Pedregal de San Angel, south-west) in Mexico City were analysed. The organic extracts were from airborne particles equal or smaller than 10 mum (PM10), and from total suspended particles (TSP). These organic extracts were assayed in the somatic mutation and recombination test (SMART) in wings of Drosophila melanogaster using two different crosses a s the genotoxic activities were higher on 02 August than on 27 July 1991 for both locations. Theamounts of airborne particles and the resulting genotoxic activities were higher at Merced than at Pedregal. In both biological systems, PM10 were more genotoxic than TSP. These results demonstrate the sensitivity of the Drosophila wing SMART - which is an in vivo eukaryotic genotoxicity assay - as a biological monitor of environmental pollution related to airborne particles.

Dobrovolsky VN, Chen T, Heflich RH. **Molecular analysis of in vivo mutations induced by N-ethyl-N-nitrosourea in the autosomal Tk and the X-linked Hprt genes of mouse lymphocytes.** Environ Mol Mutagen 1999;34(1):30-8.
The endogenous, autosomal Tk gene is a potentially useful reporter of in vivo mutation since it may recover a wider range of mutational events than the X-linked Hprt gene or bacterial transgenes. In this study, we characterized mutations produced in the Tk gene of Tk(+/-) mice and compared them with mutations induced in the Hprt gene. Treatment of Tk(+/-) mice with N-ethyl-N-nitrosourea (ENU) resulted in dose-related increases in Tk mutants, as measured by the frequency of 5-bromodeoxyuridine-resistant (BrdUrd(r)) spleen lymphocytes. ENU-induced mutant frequencies in the Hprt gene, determined by measuring 6-thioguanine-resistant (TG(r)) lymphocytes, were similar to the Tk mutant frequencies. Allele-specific PCR of DNA from BrdUrd(r) lymphocyte clones suggested that 35% of clones from mice treated with ENU and 65% of clones from untreated animals had loss of heterozygosity (LOH) of the Tk gene due to deletion of the functional Tk allele. Reverse transcriptase-PCR/sequencing analysis of BrdUrd(r) and TG(r) clones from ENU-treated mice indicated that point mutations in both genes predominantly occurred at A:T basepairs; however, A:T-->G:C transition was the most common mutation in the Tk gene, while A:T-->T:A transversion was the most frequent mutation in the Hprt gene. Substitution at A:T basepairs in the Hprt gene occurred disproportionately with the mutated dT on the nontranscribed DNA strand, while this strand bias for mutation was not seen in the Tk gene. The results indicate that the specificity of ENU-induced point mutation differs between the two endogenous genes and that the autosomal Tk gene of Tk(+/-) mice is capable of recovering mutations caused by LOH. Environ. Mol. Mutagen. 34:30-38, 1999 Published 1999 Wiley-Liss, Inc.

Edenharder R, Worf-Wandelburg A, Decker M, Platt KL. **Antimutagenic effects and possible mechanisms of action of vitamins and related compounds against genotoxic heterocyclic amines from cooked food.** Mutat Res 1999;444(1):235-48.
Possible antimutagenic activity of 26 vitamins and related compounds - ascorbic acid, beta-carotene,

cyanocobalamin, folic acid, nicotinic acid, nicotinamide, pantothenic acid, pyridoxale, pyridoxamine, pyridoxine, retinal, retinol, retinoic acid, retinyl acetate, retinyl palmitate, riboflavin, riboflavin 5'-phosphate, flavin adenine dinucleotide (FAD), alpha-tocopherol, alpha-tocopherol acetate, vitamins K (1), K(3), K(4), 1, 4-naphthoquinone, and coenzyme Q(10) - was tested against six heterocyclic amine (HCA) mutagens, i.e., 2-amino-3-methyl-imidazo[4, 5-f]quinoline (IQ), 2-amino-3,4-dimethyl-imidazo [4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-6-methyl-dipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) in the Salmonella/reversion assay using tester strains Salmonella typhimurium TA 98 and TA 100. Retinol, retinal, riboflavin, riboflavin 5'-phosphate, FAD, vitamins K(1), K(3), K(4), 1, 4-naphthoquinone, and coenzyme Q(10) caused a concentration-dependent decrease in the mutagenicity of all six mutagens in both tester strains. Quantification of antimutagenic potencies by calculating ID(50)1000; vitamin K(1): 401-740; vitamin K(3) (menadione): 85-590; vitamin K(4): 45-313; 1,4-naphthoquinone: 170-290; coenzyme Q(10): 490-860. In general, there were no major differences between HCAs tested except in part with Trp-P-2 nor between the two tester strains. In enzyme kinetic experiments with Salmonella, retinol, vitamins K(3), and K(4) behaved as competitive inhibitors of IQ induced mutagenesis. However, at the highest concentration of menadione (200 nmol/plate) and of riboflavin 5'-phosphate (2000 nmol/plate), non-competitive inhibition was observed. At other concentrations of riboflavin 5'-phosphate and at all concentrations of FAD, meaningful interpretation of enzyme kinetics were not possible. Reduction of the activity of 7-ethoxy- and 7-methoxyresorufin-O-dealkylases with IC(50) values of 2.03-30.8 microM indicated strong inhibition of 1A1 and 1A2 dependent monooxygenases by menadione and retinol. Riboflavin 5'-phosphate and FAD were less effective (IC(50): 110-803.7 microM). Nicotinamide-adenine-dinucleotidephosphate (NADPH) cytochrome P-450 reductase was not affected by retinoids but stimulated by naphthoquinones and both riboflavin derivatives up to about 50 and 80%, respectively. Again, the mutagenic activity of N-hydroxy-2-amino-3-methyl-imidazo[4,5-f]quinoline (N-OH-IQ) in Salmonella was not suppressed by K-vitamins but marginally reduced by retinol, retinal, and FAD but distinctly by riboflavin 5'-phosphate. In various experiments designed for modulation of the mutagenic response, inhibition of metabolic activation of IQ to N-OH-IQ was found to be the only relevant mechanism of antimutagenesis of menadione while a weak contribution of an other way seemed possible for retinol and FAD.

Ekwall B. **Overview of the final MEIC results: II. The in vitro-in vivo evaluation, including the selection of a practical battery of cell tests for prediction of acute lethal blood concentrations in humans.** Toxicol In Vitro 1999;13(4-5):665-73.

BIOSIS COPYRIGHT: BIOL ABS. In MEIC, all 50 reference chemicals were tested in 61 in vitro assays. To provide a background to the in vitro/in vivo evaluation, mouse LD50 values were compared with human lethal doses, resulting in a good correlation (R2 0.65). To study the relevance of in vitro results, IC50 values were compared with human lethal blood concentrations (LCs) by linear regression. An average IC50 for the ten 24-hour human cell line tests predicted peak LCs better (R2 0.74) than other groups of tests. When IC50 ls entering the brain induced a CNS depression, explaining this syndrome as a cytotoxiceffect. Multivariate analysis was used to select an optimal combination of assays, resulting in a battery of three 24-hour human cell line tests (endpoints: protein, ATP and morphology/pH) with good direct prediction of human peak LCs (R2 0.77).

Fujii S, Akiyama M, Aoki K, Sugaya Y, Higuchi K, Hiraoka M, Miki Y, Saitoh N, Yoshiyama K, Ihara K, et al. **DNA replication errors produced by the replicative apparatus of Escherichia coli.** J Mol Biol 1999;289(4):835-50.
It has been hard to detect forward mutations generated during DNA synthesis in vitro by replicative DNA polymerases, because of their extremely high fidelity and a high background level of pre-existing mutations in the single-stranded template DNA used. Using the oriC plasmid DNA replication in vitro system and the rpsL forward mutation assay, we examined the fidelity of DNA replication catalyzed by the replicative apparatus of Escherichia coli. Upon DNA synthesis by the fully reconstituted system, the frequency of rpsL-mutations in the product DNA was increased to 1.9x10(-4), 50-fold higher than the background level of the template DNA. Among the mutations generated in vitro, single-base frameshifts predominated and occurred with a pattern similar to those induced in mismatch-repair deficient E. coli cells, indicating that the major replication error was slippage at runs of the same nucleotide. Large deletions and other structural alterations of DNA appeared to be induced also during the action of the replicative apparatus. Copyright 1999 Academic Press.

Galderisi U, Di Bernardo G, Melone MA, Galano G, Cascino A, Giordano A, Cipollaro M. **Antisense inhibitory effect: a comparison between 3'-partial and full phosphorothioate antisense oligonucleotides.** J Cell Biochem 1999;74(1):31-7.
Phosphorothioate (PS) antisense oligonucleotides are currently used to inhibit many cell functions both in vivo and in vitro. However, these modified oligos provide reasonable sequence specificity only within a narrow concentration range. To overcome such a limitation we synthesized antisense oligomers, partially phosphorothioated, targeted against the human N-myc mRNA. We utilized such modified oligomers in a human neuroblastoma cell line where the N-myc gene expression was very high, and compared them to full phosphorothioate oligonucleotides. Both full PS and partial PS antisense oligos produced a maximum reduction in target mRNA after 6 h of treatment. They were able to maintain a good level of inhibition for 20 h only at high concentration. While partial PS oligos produced a dose dependent and sequence specific inhibition of N-myc mRNA, full PS molecules suffer from some disadvantages at the highest concentration used. Our results showed that partial PS molecules were capable of reducing gene expression showing a greater sequence specificity over a far broader concentration range. For this reason we conclude that partial PS antisense oligos, with respect to full PS antisense oligos, might be particularly useful for studying gene function.

Garcia Franco S, Dominguez G, Pico JC. **Alternatives in the induction and preparation of phenobarbital/naphthoflavone-induced S9 and their activation profiles.** Mutagenesis 1999;14 (3):323-6.
With the aim of optimizing the efficiency of S9 fractions used in in vitro mutagenicity assays, different schemes for the induction of liver enzymes in rats were tried and the amount of S9 fraction required was assessed. The activity of 2-anthramine (2AA), 2-acetylaminofluorene (2AAF), 3-methylcholanthrene (3MTCL) and benzo[a]pyrene in bacterial mutagenicity tests was compared with the enzymatic activity in S9 fractions obtained from rats treated with either phenobarbital (NaPB), beta-naphthoflavone (betaNF) or combinations of both. Three pool systems prepared with different amounts of NaPB-induced S9 and betaNF-induced S9 were also analyzed for their activation capacities. Profiles of standard plate incorporation assays with Salmonella typhimurium TA98 increased with the amount of S9

fraction added for all drugs tested, except for 2AA, which showed a maximun of activity at low protein concentrations. According to these profiles, an optimal S9 protein content of 700-1000 microg/plate was estimated. For 2AAF and 3MTCL an S9 fraction obtained following a simultaneous treatment with NaPB (i.p.) and betaNF (oral gavage) (NaPB + betaNF) yielded the greatest response. This preparation was the only one which produced positive activation with 3MTCL as test drug. With the other test drugs all the S9 fractions were very active, including the NAPB + betaNF-induced S9. Both Phase I and Phase II cytochrome P450 enzymatic activities were enhanced in this S9 fraction. These results suggest that the simultaneous treatment (NaPB + betaNF) would be an adequate inducer for in vitro activation when used at 700-1000 microg protein/plate.

Gichner T, Ptacek O, Stavreva DA, Plewa MJ. **Comparison of DNA damage in plants as measured by single cell gel electrophoresis and somatic leaf mutations induced by monofunctional alkylating agents.** Environ Mol Mutagen 1999;33(4):279-86.
The use of single cell gel electrophoresis (SCGE) has recently been applied to plant systems. We optimized the experimental conditions for SCGE analysis using nuclei isolated from different tissues of intact plants. Concentration-response curves of genomic DNA migration were analyzed in intact plants treated with the monofunctional alkylating agents ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), N-ethyl-N-nitrosourea (ENU), and N-methyl-N-nitrosourea (MNU). These data were used to calibrate SCGE tail moment values to induced somatic mutation in plant leaves. We used a genotoxicity index to compare genomic DNA damage and the induction of somatic mutation in the leaf tissues. The rank order of the genotoxic potency of these alkylating agents assayed by SCGE was MNU >> MMS > ENU > EMS. The rank order for the mutagenic potency of these agents was MNU >> ENU congruent with MMS > EMS. The data demonstrate the utility of SCGE analysis in plant systems. The use of SCGE will permit a larger range of plants for use as in situ environmental monitors. Also, this approach may be used to search for crop plant germplasm accessions with enhanced genomic stability. We investigated whether the intragenomic distributions of DNA damage induced by these alkylating agents were uniform and random. When a plot of the ratio of the %tail DNA and tail length versus the concentration of the test mutagen was generated, the induced SCGE data deviated from a random distribution of genomic DNA damage. Copyright 1999 Wiley-Liss, Inc.

Gilchrist R, Lomax ME, Camplejohn RS. **The need for dynamic methods for measuring cell cycle perturbations: a study in radiation-treated lymphoblastoid cell lines of varying p53 status.** Cell Prolif 1999;32(1):15-24.
Reports on the p53-related cell cycle and apoptotic responses of EBV-transformed lymphoblastoid cell lines to DNA damage have led to some confusion. This may be due to differences in the nature of the specific p53 mutations under examination, but it can also be partly attributed to methodological and analytical problems (e.g. the inappropriate use of static DNA histograms for cell cycle analysis). Taking seven lymphoblastoid cell lines derived from both normal individuals and Li-Fraumeni Syndrome/Li-Fraumeni-Like (LFS/LFL) patients of differing p53 status, we completed a detailed study of radiation-induced cell cycle perturbations. Using BrdUrd pulse labelling and flow cytometry it was found that, regardless of p53 status, the cells did not arrest in G1 despite all of the lines showing p53 upregulation 3 hours postirradiation. The irradiated cells did, however, show a general slowing both in S-phase entry from G1 and in movement through S-phase. These facts would not have been apparent from the analysis

of static DNA histograms. The problems with the use of static methods to assess changes in the dynamics of cell cycle progression apply not only to studies involving EBV-transformed cell lines, but also to a wide range of investigations into the molecular control of cell proliferation.

Gobert C, Skladanowski A, Larsen AK. **The interaction between p53 and DNA topoisomerase I is regulated differently in cells with wild-type and mutant p53.** Proc Natl Acad Sci U S A 1999;96 (18):10355-60.
DNA topoisomerase I is a nuclear enzyme involved in transcription, recombination, and DNA damage recognition. Previous studies have shown that topoisomerase I interacts directly with the tumor-suppressor protein p53. p53 is a transcription factor that activates certain genes through binding to specific DNA sequences. We now report that topoisomerase I can be stimulated by both latent and activated wild-type p53 as well as by several mutant and truncated p53 proteins in vitro, indicating that sequence-specific DNA-binding and stimulation of topoisomerase I are distinct properties of p53. These assays also suggest that the binding site for topoisomerase I on p53 is between amino acids 302 and 321. In living cells, the interaction between p53 and topoisomerase I is strongly dependent on p53 status. In MCF-7 cells, which have wild-type p53, the association between the two proteins is tightly regulated in a spatial and temporal manner and takes place only during brief periods of genotoxic stress. In marked contrast, the two proteins are constitutively associated in HT-29 cells, which have mutant p53. These findings have important implications for both cellular stress response and genomic stability, given the ability of topoisomerase I to recognize DNA lesions as well as to cause illegitimate recombination.

Godard T, Fessard V, Huet S, Mourot A, Deslandes E, Pottier D, Hyrien O, Sichel F, Gauduchon P, Poul J. **Comparative in vitro and in vivo assessment of genotoxic effects of etoposide and chlorothalonil by the comet assay.** Mutat Res 1999;444(1):103-16.
The alkaline single cell gel electrophoresis (comet) assay was used to assess in vitro and in vivo genotoxicity of etoposide, a topoisomerase II inhibitor known to induce DNA strand breaks, and chlorothalonil, a fungicide widely used in agriculture. For in vivo studies, rats were sacrificed at various times after treatment and the induction of DNA strand breaks was assessed in whole blood, bone marrow, thymus, liver, kidney cortex and in the distal part of the intestine. One hour after injection, etoposide induced DNA damage in all organs studied except kidney, especially in bone marrow, thymus (presence of HDC) and whole blood. As observed during in vitro comet assay on Chinese hamster ovary (CHO) cells, dose- and time-dependent DNA effects occurred in vivo with a complete disappearance of damage 24 h after administration. Even though apoptotic cells were detected in vitro 48 h after cell exposure to etoposide, such a result was not found in vivo. After chlorothalonil treatment, no DNA strand breaks were observed in rat organs whereas a clear dose-related DNA damage was observed in vitro. The discrepancy between in vivo and in vitro models could be explained by metabolic and mechanistic reasons. Our results show that the in vivo comet assay is able to detect the target organs of etoposide and suggest that chlorothalonil is devoid of appreciable in vivo genotoxic activity under the protocol used.

Grimaldi KA, Bingham JP, Hartley JA. **PCR-based assays for strand-specific measurement of DNA damage and repair. I. Strand-specific quantitative PCR.** Methods Mol Biol 1999;113:227-40.

Hahn A, Hock B. **Assessment of DNA damage in filamentous fungi by single cell gel**

electrophoresis, comet assay. Environ Toxicol Chem 1999;18(7):1421-4.
BIOSIS COPYRIGHT: BIOL ABS. The single cell gel electrophoresis (SCGE) assay, or comet assay, is a highly sensitive method to measure DNA damage. The principle of the assay is based on the conversion of single-strand breaks and alkali-labile sites to double-strand breaks via alkaline lysis. The cells are subjected to electrophoresis, stained, and microscopically evaluated. Increasing damage leads to increasing migration of DNA from the nuclei. Experiments were conducted with Sordaria macrospora grown for 3 d directly on ag a wide spectrum of DNA-damaging effects.

Halkiotis K, Yova D, Pantelias G. **In vitro evaluation of the genotoxic and clastogenic potential of photodynamic therapy.** Mutagenesis 1999;14(2):193-8.
Photodynamic therapy (PDT) was recently introduced in clinical practice for the management of cancer. As far as PDT relies on the combined action of a photosensitizer and a laser source, there is a need to evaluate the genotoxic and mutagenic potential of this treatment modality. This paper reports the effects of various photosensitizer and photo-irradiation doses on lethality to the MIA PaCa cell line using ZnPcS4 as the photosensitizer. The sister chromatid exchange (SCE) assay was used to evaluate the genotoxicity of various photosensitizer and photo-irradiation doses. Also, chromosomal aberrations at various time intervals post-irradiation were evaluated. The results showed that a combination of 3 J/cm2 irradiance with 5 microM ZnPcS4 concentration leads to the LD90 72 h post-irradiation. Eight days post-irradiation the LD90 level was achieved using a light dose of 3 J/cm2, independent of ZnPcS4 concentration. The SCE assay showed that cells treated with various light and drug doses presented no genotoxic potential, as SCE levels were not different from untreated (control) cells. Chromosomal analysis after PDT treatment at various time intervals post-irradiation showed that there was no significant chromosomal damage in cells treated photodynamically compared with untreated controls. The results show that the cell killing mechanism after PDT is not at the chromosome level, but may be at a different cellular level, such as plasma membranes, mitochondria, etc.

Honma M, Zhang LZ, Sakamoto H, Ozaki M, Takeshita K, Momose M, Hayashi M, Sofuni T. **The need for long-term treatment in the mouse lymphoma assay.** Mutagenesis 1999;14(1):23-9.
The L5178Y tk +/- mouse lymphoma assay (MLA) has been widely used as a genotoxicity test for the detection of mutagens and clastogens. The standard MLA, as well as other mammalian cell gene mutation assays, usually employs a short treatment period (3-6 h). Our previous report, however, suggested that such short treatments may be insufficient for detecting some clastogens and spindle poisons. For the present study, we introduced and evaluated a longer treatment (24 h) in the MLA. We examined 15 chemicals which were evaluated as negative or inconclusive in the short-term study. Cells were exposed to the chemical for 24 h without S9 mix, cultured for 2 days and then thymidine kinase-deficient mutants were selected in 96-well microtiter plates under trifluorothymidine. Eleven chemicals yielded positive responses in the 24 h treatment MLA. They included nucleoside analogs (2'-deoxycoformycin and dideoxycytidine), a base analog (1,3-dimethylxanthine) and spindle poisons (colchicine and vinblastine sulfate), all of which do not directly affect DNA, but bring about mutations and chromosome alterations through nucleoside metabolism and chromosome segregation. Because the mutagenicities of these non-DNA targeting chemicals appear to be cell cycle dependent, treatment extending over more than one cell cycle may be required for their effect. Combining results from the present and previous studies, 31 of 34 (91%) chromosome aberration-positive chemicals exhibited

positive responses in the MLA, suggesting that the sensitivity of the MLA with 24 h treatment periods approaches that of the chromosome aberration test.

Isaenko OA, Shvartsman PIA. **[Genetic effects of mitotic poisons and their modification by heat-shock in strains of Drosophila melanogaster having defective adaptive response]**. Genetika 1999;35 (5):619-30. (Rus)
Teratogenic effect of two mitotic poisons, griseofulvin and colchicine, was confirmed. A similar effect of another antimitotic agent, vinblastin, was demonstrated. The teratogenic effect of these poisons is expressed as a reduction of ommatidia in adult flies when the drug is fed to larvae. The highest frequency of phenocopies was recorded in temperature- and mutagen-sensitive strains. The mutagenic activity of vinblastin and griseofulvin was confirmed by the wing-spot test (somatic mutation and recombination test, SMART) in Drosophila melanogaster. In addition, this test demonstrated mutagenic activity of colchicine. All of the mitotic poisons induced small single spots but did not increase frequency of twin spots mwh/flr. Spot frequency was significantly higher in mutagen-sensitive mutants having defective excision repair. Heat shock (45-min exposure at 37 degrees C) decreased the frequency of phenocopies induced by the mitotic poisons. When third-instar larvae were subjected to heat shock prior to drug administration, the frequency of mutant cell clones was significantly reduced. These results indicate participation of heat-shock proteins in the protection of microtubules in actively proliferating cells of D. melanogaster.

Jenkins GJ, Takahashi N, Parry JM. **A study of ENU-induced mutagenesis in the mouse using the restriction site mutation (RSM) assay.** Teratog Carcinog Mutagen 1999;19(4):281-92.
We report here the application of the restriction site mutation (RSM) assay to study the induction of mutations by the alkylating agent ENU. Specifically, mutations were sought in the spleen and bone marrow of mice 3, 10, and 100 days after being treated with ENU; this was compared to data previously published from our laboratory on ENU-induced testes mutations. It was found that the ENU-induced mutations were all at GC bases implicating the O(6)-ethylguanosine adduct. The mutations detected reached a peak at day 10 in the spleen and were detectable to a lesser extent at 100 days, which is similar to the testes data. In the bone marrow, the mutation level rose until day 100, although the level remained below that of the spleen and testes. However, by studying the mutations detected in control animals, it was found that spontaneous mutational events were detectable at the day 100 time point in all three tissues. Hence the spleen, testes, and bone marrow mutations at day 100 in the ENU-treated samples were probably spontaneous mutational events with very few genuine ENU-induced mutations remaining in any of these tissues after 100 days. This paper also demonstrates the applicability of the inverse RSM methodology in the detection of ENU-induced mutations, whereby mutations can be detected by the conversion of one restriction site to another. The iRSM assay appears to be particularly suitable to studying alkylating agents due to their known sequence specific mutation induction. We also show a comparison of the bone marrow micronucleus data with the RSM assay and show that both assays are capable of detecting the genotoxicity of ENU to the mouse bone marrow in vivo. Copyright 1999 Wiley-Liss, Inc.

Kaya B, Yanikoglu A, Marcos R. **Genotoxicity studies on the phenoxyacetates 2,4-D and 4-CPA in the Drosophila wing spot test.** Teratog Carcinog Mutagen 1999;19(4):305-12.
The phenoxyacetates 2,4-D and 4-CPA were evaluated for genotoxicity using the Drosophila

melanogaster wing spot test, which assesses for somatic mutation and recombination events. Third-instar larvae trans-heterozygous for two recessive mutations affecting the expression of wing trichomes, multiple wing hairs (mwh), and flare (flr) were treated by chronic feeding with different concentrations of the two chemicals. Feeding lasted until pupation of the surviving larvae and the genotoxic effects induced were evaluated in adults for the appearance of wing-blade cell clones with the mwh, flr, or mwh-flr phenotypes. Exposure to 2,4-D, at the highest concentration evaluated (10 mM), induced a weak but significant increase in the frequency of two of the categories of recorded spots: large single and total spots; in contrast, the 4-CPA treatments failed to induce any significant increase in the frequency of evaluated spots. When the heterozygous larvae for mwh and the multiple inverted TM3 balancer chromosome were treated with the chemicals, no increases were detected, either after the 2,4-D nor the 4-CPA treatments. Copyright 1999 Wiley-Liss, Inc.

Keane MJ, Stephens JW, Zhong BZ, Miller WE, Ong TM, Wallace WE. **A study of the effect of chrysotile fiber surface composition on genotoxicity in vitro.** J Toxicol Environ Health 1999;57 (8):529-41.
Chrysotile fibers (NIEHS intermediate length) were treated with ultrapure HCl to alter the fiber surface chemistry without substantially changing fiber morphology or dimensions. The objective of the study was to determine whether fiber surface chemistry is an important variable in fiber genotoxicity in vitro. The modified fibers, along with native chrysotile fibers, were used to challenge Chinese hamster lung fibroblasts (V79) in vitro using the micronucleus induction genotoxicity assay. Fiber dimensions were assessed using scanning electron microscopy by measuring the distribution of fiber lengths in 3 length ranges: less than 3 microm, 3-10 microm, and greater than 10 microm. For both treated and native fiber samples, 500 fibers were examined. Results indicate that acid-treated fibers were about 20% shorter than untreated chrysotile. Surface chemistry alterations were verified by zeta-potential reversal, x-ray photoelectron spectroscopy (XPS), and scanning electron microscopy/energy-dispersive x-ray spectroscopy (SEM-EDS) elemental analysis. Scanning Auger spectrometry indicated the presence of Mg, O, and Si in both treated and native chrysotile samples, which confirmed the surface purity of both fiber samples. Both XPS and SEM-EDS analysis demonstrated substantial depletion of Mg from fiber surfaces. Results of the micronucleus assay showed a positive concentration-related response for both samples, with toxicity evident only at the highest concentration. No significant difference was found for the treated and untreated chrysotile samples. These results indicate that the surface chemistry is not an important variable in the in vitro genotoxicity of chrysotile asbestos in V79 cells as detected by the micronucleus assay under the conditions used in this study, and support a model of chemically nonspecific chromosomal and spindle damage effects.

Kim HY, Stermitz FR, Li JK, Coulombe RA Jr. **Comparative DNA cross-linking by activated pyrrolizidine alkaloids.** Food Chem Toxicol 1999;37(6):619-25.
The toxicity and bioactivity of pyrrolizidine alkaloids (PAs), common constituents of hundreds of plant species, and in herbal remedies and folk medicines prepared thereof, are probably due to their ability to form DNA cross-linking. We investigated DNA cross-linking activity by chemically-activated PAs from four different structural classes in Madin-Darby bovine kidney (MDBK) cells and in pBR322 DNA. In cell culture, alpha,beta-unsaturated macrocyclic diester pyrroles dehydrosenecionine (DHSN), dehydroriddelliine (DHRD) and the saturated macrocyclic diester pyrrole dehydromonocrotaline

(DHMO) were significantly more potent cross-linkers than the simple necine base (retronecine) and an N-oxide (indicine N-oxide; INO) as determined by alkaline elution. The proportion of total DNA cross-links that were proteinase K-resistant (DNA-DNA cross-links) induced by the various pyrroles ranged from 0.08 (DHRN) to 0.67 (DHSN). Those pyrroles that were potent cross-linkers of cellular DNA also cross-linked, in a dose-dependent manner, Bam HI-digested pBR322 DNA as assessed by a gel retardation assay. The possible functional relevance of pyrrole-DNA cross-links was determined by their ability to interrupt PCR amplification of a 1.129 kb segment of pBR322. Dehydrosenecionine completely inhibited amplification, while DHMO was of intermediate potency, while DHRN and INO had no effect. Taken together, these studies suggest that structural features, most notably the presence of a macrocyclic diester, confer potent cross-link activity to PAs. In any event, DNA-DNA cross-linking is probably biologically relevant as indicated by their interference with DNA replication.

Kindzelskii AL, Petty HR. **Ultrasensitive detection of hydrogen peroxide-mediated DNA damage after alkaline single cell gel electrophoresis using occultation microscopy and TUNEL labeling.** Mutat Res 1999;426(1):11-22.
DNA damage at the level of individual cells can be detected using the single cell gel electrophoresis (SCGE) or 'comet' assay. In the present study, we report novel variations on the conventional comet assay that can be used to enhance the microscopic detection of DNA damage. Hydrogen peroxide-treated peripheral blood leukocytes were used as a DNA damage model system. Cells were embedded in agarose, treated, and electrophoresed according to the procedure of Singh et al. [N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988), p. 184-191]. However, sites of strand breaks were directly labeled with the TUNEL (TdT-mediated fluorescein-dUTP nick end labeling) method. This labeling protocol revealed clumps and/or a series of stripes in the comet tail perpendicular to the direction of electrophoresis; these sites may account for the substructure seen in conventional comet assays. In a second comet variation, we passed an opaque disk into a field-conjugated plane of the microscope near the lamp, thus occluding the nucleus' image. Nuclear occultation allows the intensified charge-coupled device (ICCD) camera gain to increase to a single photon detection level thus revealing low levels of DNA damage in the tail. These methods offer a substantial improvement in sensitivity. Copyright 1999 Elsevier Science B.V.

Kohlpoth M, Rusche B, Nusse M. **Flow cytometric measurement of micronuclei induced in a permanent fish cell line as a possible screening test for the genotoxicity of industrial waste waters.** Mutagenesis 1999;14(4):397-402.
An in vitro micronucleus assay using the permanent fish cell line RTG-2 (rainbow trout gonads) was developed to test industrial waste waters for their genotoxic potential. Comparison of flow cytometric measurement and microscopic scoring of micronucleus frequency with the reference chemicals 1,4-butane sultone (0.2-1 mM), ethylmethane sulphonate (2-10 mM), potassium dichromate (20-100 microM) and benzo[a]pyrene (5-25 microM) showed similar dose-effect relationships. Thirty-eight industrial waste waters from 11 different branches of industry obtained from the Bavarian state office for water research were tested using the flow cytometric method (18 from metal processing, 10 from combined waste water, two from synthetic fibre production, one sample each from settlement wastes, non-iron metal manufacturing, leather production, sulphuric acid production, ore processing, graphite

film production, cellulose production and flue gas washing). Fourteen of them showed a significant increase in micronucleus frequency.

Lachance B, Robidoux PY, Hawari J, Ampleman G, Thiboutot S, Sunahara GI. **Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro.** Mutat Res 1999;444(1):25-39.
The mutagenicity and toxicity of energetic compounds such as 2,4, 6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), hexahydro-1,3, 5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3, 5,7-tetrazocine (HMX), and of amino/nitro derivatives of toluene were investigated in vitro. Mutagenicity was evaluated with the Salmonella fluctuation test (FT) and the V79 Chinese hamster lung cell mutagenicity assay. Cytotoxicity was evaluated using V79 and TK6 human lymphoblastic cells. For the TK6 and V79 assays, TNB and 2, 4,6-triaminotoluene were more toxic than TNT, whereas RDX and HMX were without effect at their maximal aqueous solubility limits. The primary TNT metabolites (2-amino-4,6-dinitrotoluene, 4-amino-2, 6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2, 6-diamino-4-nitrotoluene) were generally less cytotoxic than the parent compound. The FT results indicated that TNB, TNT and all the tested primary TNT metabolites were mutagenic. Except for the cases of 4-amino-2,6-dinitrotoluene and 2,4-diamino-6-nitrotoluene in the TA98 strain, addition of rat liver S9 resulted in either no effect, or decreased activity. None of the tested compounds were mutagenic for the V79 mammalian cells with or without S9 metabolic activation. Thus, the FT assay was more sensitive to the genotoxic effects of energetic compounds than was the V79 test, suggesting that the FT might be a better screening tool for the presence of these explosives. The lack of mutagenicity of pure substances for V79 cells under the conditions used in this study does not preclude that genotoxicity could actually exist in other mammalian cells. In view of earlier reports and this study, mutagenicity testing of environmental samples should be considered as part of the hazard assessment of sites contaminated by TNT and related products.

Le Curieux F, Nesslany F, Munter T, Kronberg L, Marzin D. **Genotoxic activity of chlorohydroxyfuranones in the microscale micronucleus test on mouse lymphoma cells and the unscheduled DNA synthesis assay in rat hepatocytes.** Mutagenesis 1999;14(5):457-62.
BIOSIS COPYRIGHT: BIOL ABS. Chlorohydroxyfuranones (CHFs) are mutagenic disinfection by-products found in chlorine-treated drinking water. In the current study, the genotoxicity of four CHFs, 3,4-dichloro-5-hydroxy-2(5H)-furanone (MCA), 3-chloro-4-methyl-5-hydroxy-2(5H)-furanone (MCF), 3-chloro-4-(chloromethyl)-5-hydroxy-2(5H)-furanone (CMCF) and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), was determined. Two in vitro assays, the microscale micronucleus assay on L5178Y mouse lymphoma cells and the unschedule e range of genetic damages induced by this group of compounds.

Leblanc GA, Bain LJ. **Chronic toxicity of environmental contaminants: sentinels and biomarkers.** Environ Health Perspect 1997 Feb;105 Suppl 1:65-80.
Due to the use of a limited number of species and subchronic exposures, current ecological hazard assessment processes can underestimate the chronic toxicity of environmental contaminants resulting in adverse responses of sentinel species. Several incidences where sentinel species have responded to the effects of chronic exposure to ambient levels of environmental contaminants are discussed, including the development of neoplasia in fish, immunosuppression in marine mammals, pseudohermaphrodism in

invertebrates, teratogenicity in amphibians, and aberrations in the sexual development of fish and reptiles. Biomarkers of chronic toxicity, including DNA mutations, alterations in specific protein and mRNA levels, and perturbations in metabolism, are presented. The incorporation of appropriate surrogate species and biomarkers of chronic toxicity into standard toxicity characterizations is proposed as a means of significantly refining the ecological hazard assessment process.

Lovell DP, Thomas G, Dubow R. **Issues related to the experimental design and subsequent statistical analysis of in vivo and in vitro comet studies.** Teratog Carcinog Mutagen 1999;19(2):109-19.
A wide range of experimental designs are used in investigations using the Comet assay. The statistical issues associated with this assay are however not particularly unusual or difficult. It is important however to recognize that the sample rather than the cell is the experimental unit. Statistical analyses based upon measures from the individual cells can lead to serious misinterpretation of results. Interpretation of the results of the assay should be related to identifying changes of biological importance rather than relying solely on the P values of specific statistical tests.

Lowes DA, Brown K, Heydon RT, Martin EA, Gant TW. **Site-specific tamoxifen-DNA adduct formation: lack of correlation with mutational ability in Escherichia coli.** Biochemistry 1999;38 (34):10989-96.
We have mapped sites of tamoxifen adduct formation, in the lacI gene using the polymerase STOP assay, following reaction in vitro with alpha-acetoxytamoxifen and horseradish peroxidase (HRP)/H(2)O (2) activated 4-hydroxytamoxifen. For both compounds, most adduct formation occurred on guanines. However, one adenine, within a run of guanines, generated a strong polymerase STOP site with activated 4-hydroxytamoxifen, and a weaker STOP site with alpha-acetoxytamoxifen at the same location. In Escherichia coli the lac I gene reacted with 4-hydroxytamoxifen was more likely to be mutated (2 orders of magnitude) than when reacted with alpha-acetoxytamoxifen, despite the greater DNA adduct formation by alpha-acetoxytamoxifen. This correlates with the greater predicted ability of activated 4-hydroxytamoxifen adducts to disrupt DNA structure than alpha-acetoxytamoxifen adducts. For lac I reacted with activated 4-hydroxytamoxifen, a hot spot of base mutation was located in the region of the only adenosine adduct. No mutational hot spots were observed with alpha-acetoxytamoxifen. Our data clearly shows a lack of correlation between gross adduct number, as assayed by (32)P-postlabeling and mutagenic potential. These data indicate the importance of minor adduct formation in mutagenic potential and further that conclusions regarding the mutagenicity of a chemical may not be reliably derived from the gross determination of adduct formation.

Maran U, Karelson M, Katritzky AR. **A comprehensive QSAR treatment of the genotoxicity of heteroaromatic and aromatic amines.** Quant Struct Activity Relat 1999;18(1):3-10.
BIOSIS COPYRIGHT: BIOL ABS. A quantitative structure-activity relationship with R2 = 0.8344 (R = 0.9135) has been derived from a set of 95 heteroaromatic and aromatic amines to correlate and predict their mutagenic activity. It consists of six descriptors calculated from the molecular structures with quantum-chemical methods. The descriptors in the model reveal the importance in mutagenic interactions of heteroaromatic amines of hydrogen bonding, of effects induced by the solvent, and of the size of compound. The model als.

Marrot L, Belaidi JP, Meunier JR, Perez P, Agapakis-Causse C. **The human melanocyte as a particular target for UVA radiation and an endpoint for photoprotection assessment.** Photochem Photobiol 1999;69(6):686-93.
The induction of DNA breaks by UVA (320-400 nm) in the nucleus of normal human melanocytes in culture was investigated using single cell gel electrophoresis, also called the comet assay. Endogenous pigment and/or melanin-related molecules were found to enhance DNA breakage: comets were more intense in melanocytes than in fibroblasts, in cells with high melanin content or after stimulation of melanogenesis by supplying tyrosine in the culture medium. After UVA doses where strong comets were observed, neither cytotoxicity nor stimulation of tyrosinase activity were detected. However, the accumulation of p53 protein suggested that cells reacted to genotoxic stress under these experimental conditions. The same approach was used to compare two sunscreens with identical sun protection factors but different UVA protection factors. The results presented in this paper suggest that human melanocytes may be used as a target cell to evidence broadspectrum photoprotection. Moreover, these data appear to be helpful in getting a better understanding of the role of sunlight in the initiating steps of melanocyte transformation.

Matton N, Simonetti J, Williams K. **Inefficient in vivo repair of mismatches at an oncogenic hotspot correlated with lack of binding by mismatch repair proteins and with phase of the cell cycle.** Carcinogenesis 1999;20(8):1417-24.
Repair rates of mismatched nucleotides located at an activating hotspot of mutation, H-ras codon 12, have been analyzed in vivo in mammalian cells. Repair rates at codon 12 are significantly improved in cells synchronized to the G(1) stage of the mammalian cell cycle as compared with non-synchronous cells, demonstrating that mismatch repair mechanisms are active in G(1). Repair rates in non-synchronous cells for the same mismatches at a nearby non-hotspot of mutation, H-ras codon 10, are also significantly improved over repair rates at codon 12 in non-synchronous cells, demonstrating that DNA mismatch repair rates can differ depending on the sequence context. These results suggest that inefficiencies in mismatch repair are responsible, at least in part, for the well documented hotspot of mutation at codon 12. Further experiments involving gel-shift analysis demonstrate a mismatch-specific binding factor for which the degree of binding correlates with in vivo repair rates for each mismatch tested at the codon 12 location. This binding factor appears to be the hMutSalpha heterodimer as identified by monoclonal antibody assay and inhibition of binding by ATP. Furthermore, a lack of binding is observed only for G:A mismatches at the codon 12 location. This lack of binding correlates with the low rate of repair observed in vivo for G:A mismatches at codon 12 versus the improved repair rates for G:A mismatches at codon 10. This may have biological relevance in that G:C-->T:A tranversions are a common mutation at this location in naturally occurring human tumors. These results suggest that there is lowered efficiency in the kinetics of mismatch repair at codon 12. Mismatches at this location are therefore more likely to be replicated before repair, thus resulting in a mutation.

Mendonca MS, Howard K, Desmond LA, Derrow C Weissman. **Previous loss of chromosome 11 containing a suppressor locus increases radiosensitivity, neoplastic transformation frequency and delayed death in HeLa fibroblast human hybrid cells.** Mutagenesis 1999;14(5):483-9.
BIOSIS COPYRIGHT: BIOL ABS. CGL1 (HeLaells have been utilized to study mechanisms of radiation-induced neoplastic transformation of human cells in vitro. Previous analysis has shown that

loss of active tumor suppressor alleles on fibroblast chromosomes 11 and 14 may be required for radiation-induced neoplastic transformation of CGL1 cells. Loss of chromosome 11 alone was, therefore, found to be necessary but not sufficient for neoplastic transformation. We postulated that the loss of chromosome 11 ma n when compared with the parental CGL1 cells. In addition, the neoplastically transformed foci appear to arise earlier after radiation exposure in CON104(-11) versus CGL1 cells. Furthermore, the plating efficiency (PE) of the progeny of the irradiated CON104(-11) cells, growing in transformation flasks, is persistently lower than parental CGL1 cells during the 21 day assay period. The lower PE of the progeny of irradiated cells was attributed to the expression of delayed death/lethal mutations p e stability after radiation damage.

Miwa T, Emi N, Nonami T, Kurokawa T, Yoshikawa K, Nakao A, Takagi H. **Gene transfer with cationic lipid into human hepatocellular carcinoma in nude mice.** Hepatogastroenterology 1999;46 (26):825-9.
BACKGROUND/AIMS: Using a cationic lipid, gene transfection into the tumor of a human hepatocellular carcinoma model in nude mice was attempted in order to explore the possibility of its use in gene therapy. METHODOLOGY: A DNA-lipid complex was made by combining the cationic lipid distearyldimethyl ammonium bromide (DDAB) with pCMV sPORT expressing the reporter gene LacZ. The expression of this complex was first investigated in vitro against the human hepatocellular carcinoma cell line Li7HM. It was then injected directly into a hepatocellular carcinoma model tumor created by implanting Li7HM into the liver of BALB/c nu/nu mice, and the expression of LacZ was histologically evaluated. RESULTS: LacZ gene was expressed in Li7HM in vitro with the optimized DNA-lipid complex. Cell toxicity was not a problem. Expression of LacZ was also seen in the mouse hepatocellular model tumor into which the complex had been injected, indicating successful gene transfection with this method. CONCLUSIONS: Direct injection of a DNA-lipid complex into a mouse hepatocellular carcinoma model tumor is a safe and simple method of gene transfection, proving this to be a viable method of transfer for use in gene therapy.

Mori M, Kobayashi H, Sugiyama C, Katsumura Y, Furihata C. **Induction of unscheduled DNA synthesis in hairless mouse epidermis by skin carcinogens.** J Toxicol Sci 1999;24(3):217-26.
Induction of unscheduled DNA synthesis (UDS) in hairless mouse epidermis by six chemicals was determined in an in vivo-in vitro assay by using a liquid scintillation counting method. Test chemicals were applied once onto two areas of the back of female hairless mice after stripping of the stratum corneum with adhesive tape to enhance skin penetration. After exposure, the skin samples were taken and cultured in a medium containing [3H]thymidine with or without hydroxyurea (HU, an inhibitor of replicative DNA synthesis). DNA of the epidermis was extracted, and incorporation of [3H]thymidine into DNA and the DNA content was determined with a liquid scintillation counter and a fluorescence spectrophotometer, respectively. Induction of UDS by chemicals was judged by calculation of the UDS index [(the ratio of DNA synthesis in the presence of HU to that in its absence) x 100]. A good correlation between UDS induction and organ specificity of carcinogens was observed. 4-Nitroquinoline 1-oxide, a skin carcinogen used as a positive control, induced a dose-dependent increase in the UDS index of approximately 12-fold at 2 hr after exposure, while 1,2-epoxydodecane, a non-skin carcinogen applied as a negative control, did not increase the UDS index. Four other skin carcinogens induced dose-dependent increases in the UDS index; N-methyl-N'-nitro-N-nitrosoguanidine and diepoxybutane at 2 hr

after exposure, and 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene at 24 hr after exposure. The results suggest that UDS is a good marker of the genotoxicity of skin carcinogens.

Mueller SO, Stopper H. **Characterization of the genotoxicity of anthraquinones in mammalian cells.** Biochim Biophys Acta 1999;1428(2-3):406-14.
Naturally occurring 1,8-dihydroxyanthraquinones are under consideration as possible carcinogens. Here we wanted to elucidate a possible mechanism of their genotoxicity. All three tested anthraquinones, emodin, aloe-emodin, and danthron, showed capabilities to inhibit the non-covalent binding of bisbenzimide Hoechst 33342 to isolated DNA and in mouse lymphoma L5178Y cells comparable to the topoisomerase II inhibitor and intercalator m-amsacrine. In a cell-free decatenation assay, emodin exerted a stronger, danthron a similar and aloe-emodin a weaker inhibition of topoisomerase II activity than m-amsacrine. Analysis of the chromosomal extent of DNA damage induced by these anthraquinones was performed in mouse lymphoma L5178Y cells. Anthraquinone-induced mutant cell clones showed similar chromosomal lesions when compared to the topoisomerase II inhibitors etoposide and m-amsacrine, but were different from mutants induced by the DNA alkylator ethyl methanesulfonate. These data support the idea that inhibition of the catalytic activity of topoisomerase II contributes to anthraquinone-induced genotoxicity and mutagenicity.

Muller L, Kikuchi Y, Probst G, Schechtman L, Shimada H, Sofuni T, Tweats D. **ICH-harmonised guidances on genotoxicity testing of pharmaceuticals: evolution, reasoning and impact.** Mutat Res 1999;436(3):195-225.
The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has convened an expert working group which consisted of the authors of this paper and their respective committees, consulting groups and task forces. Two ICH guidances regarding genotoxicity testing have been issued: S2A, 'Guidance on Specific Aspects of Regulatory Genotoxicity Tests' and S2B, 'Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals.' Together, these guidance documents now form the regulatory backbone for genotoxicity testing and assessment of pharmaceuticals in the European Union, Japan, and the USA. These guidances do not constitute a revolutionary new approach to genotoxicity testing and assessment, instead they are an evolution from preexisting regional guidelines, guidances and technical approaches. Both guidances describe a number of specific criteria as well as a general test philosophy in genotoxicity testing. Although these guidances were previously released within the participating regions in their respective regulatory communiques, to ensure their wider distribution and better understanding, the texts of the guidances are reproduced here in their entirety (see Appendix A) and the background for the recommendations are described. The establishment of a standard battery for genotoxicity testing of pharmaceuticals was one of the most important issues of the harmonisation effort. This battery currently consists of: (i) a test for gene mutation in bacteria, (ii) an in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma tk assay, (iii) an in vivo test for chromosomal damage using rodent hematopoietic cells. A major change in testing philosophy is the acceptance of the interchangeability of testing for chromosomal aberrations in mammalian cells and the mouse lymphoma tk assay. This agreement was reached on the basis of the extensive review of databases and newly generated experimental data which are in part described in this publication. The authors are fully aware of the fact that some of the recommendations given in these ICH guidances are

transient in nature and that the dynamic qualities and ongoing evolution of genetic toxicology makes necessary a continuous maintenance process that would serve to update the guidance as necessary. Copyright 1999 Elsevier Science B.V.

Nalecz-Jawecki G, Sawicki J. **Spirotox--a new tool for testing the toxicity of volatile compounds.** Chemosphere 1999;38(14):3211-8.
A new method for estimating the toxicity of volatile compounds was developed. The test was carried out in the disposable polystyrene multiwells. After the organisms, protozoa Spirostomum ambiguum, were added to the wells, microplate was tightly closed using silicone grease and polyethylene film. The toxicities of 21 organic compounds were estimated. No control mortality was observed in all cases. Transparent PE film enabled good observation of test response. The toxicity of tested compounds varied over 4 orders of magnitude. Deformations were 2-4 more sensitive toxic response then lethality. The toxicity of tested compounds in Spirotox test correlates well with the log Kow and toxicity results from other bioassays: Microtox, D. magna and T. pyriformis.

Nesslany F, Marzin D. **A micromethod for the in vitro micronucleus assay.** Mutagenesis 1999;14 (4):403-10.
A micromethod for the in vitro micronucleus assay was developed using L5178Y cells to enable the rapid screening of a large number of molecules. The method is quick, simple to perform and needs very small amounts of compound, i.e. <10 mg. In this methodology, three types of treatment were carried out in parallel, enabling an optimal detection of both aneugenic and clastogenic compounds: two treatments without metabolic activation with or without a recovery period after a 24 h continuous treatment and one treatment with metabolic activation by Aroclor 1254-induced rat or hamster liver S9 mix. Seventeen known genotoxins (12 clastogens and five aneugens) and seven known non-genotoxins were tested. The in vitro micronucleus micromethod using L5178Y cells exhibited good sensitivity (16 positive/17 known genotoxins tested) and specificity (7 negative/7 known non-genotoxins tested) for the 24 test compounds studied with or without metabolic activation. Furthermore, this test showed a good correlation with other in vitro micronucleus tests performed using macromethods with various mammalian cell cultures. We conclude that the in vitro micronucleus micromethod with L5178Y cells could be used in the earliest stages of development of new molecules as a preliminary short-term screening assay before starting regulatory tests.

O'Brien T, Karlsen AE, Andersen HU, Mandrup-Poulsen T, Nerup J. **Absence of toxicity associated with adenoviral-mediated transfer of the beta-galactosidase reporter gene to neonatal rat islets in vitro.** Diabetes Res Clin Pract 1999;44(3):157-63.
Transfer of genes with potential therapeutic utility to the pancreatic islets of Langerhans may enhance graft survival after islet transplantation. The aim of this study was to determine the optimal conditions for adenoviral-mediated gene transfer to the islets of Langerhans in the absence of vector-induced toxicity. Neonatal rat islets were transduced in groups of 25 with an adenoviral vector encoding beta-galactosidase (AdbetaGal) at doses of MOI 0, 10, 100 and 1000 pfu per islet cell. All experiments were performed in triplicate. Efficiency of gene transfer was determined by gross inspection and estimation of the percentage of beta-galactosidase positive cells after islet dispersion at 1, 4, 7 and 10 days post-transduction. Islet toxicity was assessed by measuring accumulated insulin levels at each time-point and by assessing static incubation insulin release at 3 and 10 days. Efficient dose-dependent gene transfer to

the islets was documented at 1, 4, 7 and 10 days post-transduction. Transgene expression was relatively stable for the duration of the experiment. Insulin accumulation did not differ between transduced and non-transduced islets at each timepoint. Likewise, the insulin secretory response to glucose, obtained by dividing the insulin response to high glucose incubation by the insulin response to low glucose incubation was similar in transduced and non-transduced islets at 3 and 10 days at all doses studied. In summary, adenoviral-mediated transduction of islets results in dose dependent efficient gene transfer with relatively stable transgene expression in the absence of toxicity. This technology may be useful in the study of islet biology and also in the future in gene therapy approaches to the treatment of diabetes mellitus.

Offer H, Wolkowicz R, Matas D, Blumenstein S, Livneh Z, Rotter V. **Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery.** FEBS Lett 1999;450(3):197-204.
The p53 tumor suppressor that plays a central role in the cellular response to genotoxic stress was suggested to be associated with the DNA repair machinery which mostly involves nucleotide excision repair (NER). In the present study we show for the first time that p53 is also directly involved in base excision repair (BER). These experiments were performed with p53 temperature-sensitive (ts) mutants that were previously studied in in vivo experimental models. We report here that p53 ts mutants can also acquire wild-type activity under in vitro conditions. Using ts mutants of murine and human origin, it was observed that cell extracts overexpressing p53 exhibited an augmented BER activity measured in an in vitro assay. Depletion of p53 from the nuclear extracts abolished this enhanced activity. Together, this suggests that p53 is involved in more than one DNA repair pathway.

Olive PL. **DNA damage and repair in individual cells: applications of the comet assay in radiobiology.** Int J Radiat Biol 1999;75(4):395-405.
The comet assay is a single-cell gel electrophoresis method that can measure a variety of types of DNA damage, and repair of damage, in individual cells. It is now in widespread use in genetic toxicology and oncology. This review describes the history of the development of this method and its applications in radiation biology, with particular emphasis on the use of the comet assay to measure heterogeneity in DNA damage in cells exposed to ionizing radiation.

Ozturk K, Durusoy M. **The detection and comparison of the genotoxic effects of some nitro aromatic compounds by the umu and SOS chromotest systems.** Toxicol Lett 1999;108(1):63-8.
Four nitroarenes were tested in two standard genotoxicity assay systems using three well-known bacterial tester strains. The results were as follows: 4-nitroquinoline l-oxide (4NQO) was positive in Quillardet and Hofnung's SOS chromotest using Escherichia coli strain PQ37 both in the presence and absence of microsomal (S9) supplements and in the Salmonella typhimurium umu tester strains NM2009 and NM3009, which express high levels of O-acetyltransferase (O-AT) and O-AT plus nitroreductase (NR) respectively. m-Nitrocinnamic acid (m-NCA) was weakly positive in strains NM2009 and NM3009, but negative in the SOS chromotest; m-dinitrobenzene (m-DNB) was weakly positive in strain NM2009, intermediate positive in strain NM3009, but negative in the SOS chromotest; 2,4-dinitrotoluene (2,4-DNT) was weakly positive in strain NM3009, but negative in strain NM2009 and in the SOS chromotest. However it still showed a dose-response relationship in strain NM2009. In view of these results, it is suggested that investigators planning to screen miscellaneous nitroarenes for their genotoxicity in the future should consider taking advantage of the increased sensitivity which is

conferred on S. typhimurium strains NM2009 and NM3009 by virtue of their capacity to overexpress O-AT or O-AT and NR.

Preston RJ. **Cytogenetic effects of ethylene oxide, with an emphasis on population monitoring.** Crit Rev Toxicol 1999;29(3):263-82.
Cytogenetic assays are an integral component of the battery of short-term assays that are used for the hazard identification component of a cancer risk assessment. The protocol for the conduct of such assays for maximal sensitivity for detecting clastogenicity has to be attendant to the mechanism of induction of the endpoint being assessed and the fact that several aberration types are cell lethal necessitates that analysis be for cells at their first posttreatment metaphase. Cytogenetic assays for human populating monitoring have been used for predicting potential for carcinogenicity in humans. However, the assays as typically conducted are not appropriate for chronic exposures because nontransmissible alterations are assessed. The use of fluorescent in situ hybridization (FISH) techniques for the assessment of transmissible changes such as reciprocal translocations are required to make population monitoring studies interpretable, and for removing some of the concern over the influence of confounders on outcome. The database for the cytogenetic effects of ethylene oxide in vitro and in vivo, with an emphasis on human population monitoring, has been critically reviewed. Based on the endpoints studied, the size of the study groups, the information on exposure, the nature of any exposure response data, and the possible influence of confounders (i.e., control matching), it is concluded that acute, high exposures to ethylene oxide with sampling shortly (a few days) after exposure can be detected by increases in chromosome aberrations or SCE in peripheral lymphocytes. Such increases are indicators of exposure to a genotoxic chemical and not predictors of subsequent adverse health effects to individuals. The effect of chronic and/or low level (less than about 25 ppm) exposures cannot be reliably evaluated using current methods. The use of FISH, for example, for assessing reciprocal translocation frequencies (as a measure of transmissible events) will greatly improve the ability to detect chronic exposures to clastogenic chemicals.

Przybojewska B. **Assessment of aniline derivatives-induced DNA damage in the liver cells of B6C3F1 mice using the alkaline single cell gel electrophoresis ("comet") assay.** Teratogen Carcinogen Mutagen 1999;19(5):323-7.
BIOSIS COPYRIGHT: BIOL ABS. The alkaline single cell gel electrophoresis (SCGE) or "comet" assay under alkaline conditions was used to measure DNA damage in the liver cells of B6C3F1 male mice exposed to 2,4-dimethylaniline and 2,4,6-trimethylaniline. Cells embedded in agarose were lysed, subjected briefly to an electric field, stained with a fluorescent DNA-binding stain, and viewed using a fluorescence microscope. The effect of 2,4-dimethylaniline and 2,4,6-trimethylaniline was studied after a single intraperitoneal inje d the results obtained by other researchers who reported mutagenic activity of 2,4-dimethylaniline and 2,4,6-trimethylaniline in Salmonella typhimurium assay and in a DNA repair test using Chinese hamster hepatocytes, it can be stated that both aromatic amines are genotoxic.

Rajaguru P, Fairbairn LJ, Ashby J, Willington MA, Turner S, Woolford LA, Chinnasamy N, Rafferty JA. **Genotoxicity studies on the azo dye Direct Red 2 using the in vivo mouse bone marrow micronucleus test.** Mutat Res 1999;444(1):175-80.
The clastogenicity of the azo dye Direct Red 2 (DR2) has been investigated using the murine bone marrow micronucleus assay. A potent dose-dependent response was observed following oral gavage of

DR2 up to 4 mg/kg, after which significant toxicity to the erythroid compartment was observed. The route of administration had a significant effect on the frequency of micronucleus formation: intraperitoneal injection was approximately two-fold less clastogenic than the equivalent dose delivered orally ($p<0.05$). The requirement for activation of DR2 by intestinal microflora was indicated by the fact that mice given acid-treated water prior to administration of DR2 showed a significant reduction (40%; $p<0.001$) in micronucleated polychromatic erythrocyte formation. The implications of these findings for the health and safety of occupationally exposed workers are discussed.

Rothkamm K, Lobrich M. **Misrejoining of DNA double-strand breaks in primary and transformed human and rodent cells: a comparison between the HPRT region and other genomic locations.** Mutat Res 1999;433(3):193-205.
Many studies of radiation response and mutagenesis have been carried out with transformed human or rodent cell lines. To study whether the transfer of results between different cellular systems is justified with regard to the repair of radiation-induced DNA double-strand breaks (DSBs), two assays that measure the joining of correct DSB ends and total rejoining in specific regions of the genome were applied to primary and cancer-derived human cells and a Chinese hamster cell line. The experimental procedure involves Southern hybridization of pulsed-field gel electrophoresis blots and quantitative analysis of specific restriction fragments detected by a single-copy probe. The yield of X-ray-induced DSBs was comparable in all cell lines analyzed, amounting to about 1 x 10(-2) breaks/Mbp/Gy. For joining correct DSB ends following an 80 Gy X-ray exposure all cell lines showed similar kinetics and the same final level of correctly rejoined breaks of about 50%. Analysis of all rejoining events revealed a considerable fraction of unrejoined DSBs (15-20%) after 24 h repair incubation in the tumor cell line, 5-10% unrejoined breaks in CHO cells and complete DSB rejoining in primary human fibroblasts. To study intragenomic heterogeneity of DSB repair, we analyzed the joining of correct and incorrect break ends in regions of different gene density and activity in human cells. A comparison of the region Xq26 spanning the hypoxanthine guanine phosphoribosyl transferase locus with the region 21q21 revealed identical characteristics for the induction and repair of DSBs, suggesting that there are no large variations between Giemsa-light and Giemsa-dark chromosomal bands.

Saha N, Schwer B, Shuman S. **Characterization of human, Schizosaccharomyces pombe, and Candida albicans mRNA cap methyltransferases and complete replacement of the yeast capping apparatus by mammalian enzymes.** J Biol Chem 1999;274(23):16553-62.
Human and fission yeast cDNAs encoding mRNA (guanine-N7) methyltransferase were identified based on similarity of the human (Hcm1p; 476 amino acids) and Schizosaccharomyces pombe (Pcm1p; 389 amino acids) polypeptides to the cap methyltransferase of Saccharomyces cerevisiae (Abd1p). Expression of PCM1 or HCM1 in S. cerevisiae complemented the lethal phenotype resulting from deletion of the ABD1 gene, as did expression of the NH2-terminal deletion mutants PCM1(94-389) and HCM1(121-476). The CCM1 gene encoding Candida albicans cap methyltransferase (Ccm1p; 474 amino acids) was isolated from a C. albicans genomic library by selection for complementation of the conditional growth phenotype of S. cerevisiae abd1-ts mutants. Human cap methyltransferase was expressed in bacteria, purified, and characterized. Recombinant Hcm1p catalyzed quantitative S-adenosylmethionine-dependent conversion of GpppA-capped poly(A) to m7GpppA-capped poly(A). We identified by alanine-scanning mutagenesis eight amino acids (Asp-203, Gly-207, Asp-211, Asp-227,

Arg-239, Tyr-289, Phe-291, and Phe-354) that are essential for human cap methyltransferase function in vivo. All eight residues are conserved in other cellular cap methyltransferases. Five of the mutant human proteins (D203A, R239A, Y289A, F291A, and F354A) were expressed in bacteria and found to be defective in cap methylation in vitro. Concordance of mutational effects on Hcm1p, Abd1p, and vaccinia capping enzyme underscores a conserved structural basis for cap methylation in DNA viruses, yeast, and metazoans. This is in contrast to the structural and mechanistic divergence of the RNA triphosphatase components of the yeast and metazoan capping systems. Nevertheless, we demonstrate that the entire three-component yeast capping apparatus, consisting of RNA 5'-triphosphatase (Cet1p), RNA guanylyltransferase (Ceg1p), and Abd1p could be replaced in vivo by the two-component mammalian apparatus consisting of a bifunctional triphosphatase-guanylyltransferase Mce1p and the methyltransferase Hcm1(121-476)p. Isogenic yeast strains with fungal versus mammalian capping systems should facilitate rational screens for antifungal drugs that target cap formation in vivo.

Schiestl RH, Aubrecht J, Yap WY, Kandikonda S, Sidhom S. **Polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin induce intrachromosomal recombination in vitro and in vivo.** Cancer Res 1997 Oct 1;57(19):4378-83.
Polychlorinated aromatic hydrocarbons such as polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are extremely stable and widely distributed environmental pollutants. These chemicals are animal carcinogens and probable human carcinogens, and TCDD is possibly one of the most potent toxins ever evaluated by the United States Environmental Protection Agency. Polychlorinated aromatic hydrocarbons score negatively in most genotoxicity assays, including the Ames (Salmonella) assay. Although their mechanism of toxicity is not well understood, they induce aryl hydrocarbon (AH) hydroxylases and bind to the AH receptor, which is believed to mediate toxicity. Here, we determine effects of polychlorinated aromatic hydrocarbons in genotoxicity assays that score for DNA deletions by intrachromosomal recombination in vivo and in vitro. In this study, TCDD, Aroclor 1221, and Aroclor 1260 induced deletions in vivo in the mouse embryo; Aroclor 1221 and Aroclor 1260 induced deletions in yeast. We also show that the induced deletion events did not correlate with induction of AH hydroxylase. None of the tested compounds induced CYP1A-associated ethoxyresorufin-O-deethylase activity in mouse embryos or in vitro. These results clearly demonstrate a genotoxic activity of polychlorinated aromatic hydrocarbons in vitro and in vivo, which is independent of induction of cytochrome P450 activity. Because genetic instability and deletions may be mechanistically involved in carcinogenesis, these results may encourage further research to determine whether such genotoxic mechanisms may be useful for cancer risk assessment of polychlorinated aromatic hydrocarbons.

Schweigert N, Belkin S, Leong-Morgenthaler P, Zehnder A, Eggen R. **Combinations of chlorocatechols and heavy metals cause DNA degradation in vitro but must not result in increased mutation rates in vivo.** Environ Mol Mutagen 1999;33(3):202-10.
BIOSIS COPYRIGHT: BIOL ABS. Chlorocatechols introduced into the environment directly or as a result of degradation processes are highly toxic, particularly when combined with heavy metals. With in vitro DNA degradation assays, the high reactivity of chlorocatechols combined with heavy metals could be shown, whereby copper was shown to be more active than iron. Structure-activity analysis showed that the degradation potential of the chlorocatechols decreased with an increasing number of chloratoms.

The addition of reactive ced mutation rate. This phenomenon was explained by doing marker gene expression measurements and toxicity tests with E. coli mutants deficient in oxidative stress defense or DNA repair. In catechol-copper-exposed cultures an increased peroxide level could indeed be demonstrated, but the highly efficient defense and repair systems of E. coli avoid the phenotypical establishment of mutations. Increased mutation rates under chronic exposure, however, cannot be excluded.

Schwenzer R, Siemienski K, Liptay S, Schubert G, Peters N, Scheurich P, Schmid RM, Wajant H. **The human tumor necrosis factor (TNF) receptor-associated factor 1 gene (TRAF1) is up-regulated by cytokines of the TNF ligand family and modulates TNF-induced activation of NF-kappaB and c-Jun N-terminal kinase.** J Biol Chem 1999;274(27):19368-74.
To understand how the TNF receptor-associated factor 1 (TRAF1) is transcriptionally regulated, in vitro DNA binding assays, promoter-reporter gene assays, and RNase protection assays were performed with the human TRAF1 gene. Binding of NF-kappaB to three of five putative binding sites within the human TRAF1 promoter was found in electrophoretic mobility shift assay studies, and analysis of TRAF1 gene promoter luciferase constructs confirmed the functional importance of these elements. Moreover, triggering of TNF-R1, CD40, and the interleukin-1 receptor resulted in transcription of the TRAF1 gene, whereas receptors that are not activators or only poor activators of NF-kappaB in HeLa cells failed to show a significant TRAF1 induction. Because it has been shown that members of the TRAF family are involved in activation of NF-kappaB and the c-Jun N-terminal kinase (JNK) by the interleukin-1 receptor and members of the TNF receptor superfamily, a role of TRAF1 in receptor cross-talk and/or feedback regulation of activated receptor signaling complexes can be suggested. In fact, we found that TNF-induced activation of JNK is prolonged in transfectants overexpressing TRAF1, whereas overexpression of a deletion mutant of TRAF1 in which the N-terminal part had been replaced by the green fluorescent protein interfered with TNF-induced activation of NF-kappaB and JNK.

Snyder RD, Strekowski L. **Enhancement of bleomycin-induced micronucleus formation in V79 cells as a rapid and sensitive screen for non-covalent DNA-binding compounds.** Mutat Res 1999;444 (1):181-92.
Non-covalent drug/DNA interactions are difficult to study and because of this, the significance of such interactions from a safety standpoint and their contribution to positive genetic toxicology test findings is poorly understood. It is shown in the present study that such interactions may be detected and quantified in Chinese hamster V79 cells by an adaptation of the bleomycin amplification assay. This assay measures the ability of a test compound to enhance the DNA damaging activity of the antibiotic bleomycin using micronucleus formation as an endpoint. Results are presented examining the bleomycin amplification activity of known intercalating agents, groove-binding agents and other structurally diverse classes of compounds for which intercalative status has not been reported. The assay reveals a strong and predictable SAR for amplification activity based on number and orientation of aromatic rings. Moreover, excellent correlations are observed between DNA binding (viscometric analyses) and DNA amplification in V79 cells for a series of seven experimental compounds. The assay is shown to be useful in understanding the genotoxicity of marketed antihistamines and to help explain genetic toxicology findings observed in a series of novel pharmaceutical entities. It is proposed that assessment of bleomycin amplification activity of novel compounds in early genotoxicity prescreening may provide

important information upon which to base synthesis of compounds with minimal or no genotoxic liability.

Speit G, Hartmann A. **The comet assay (single-cell gel test). A sensitive genotoxicity test for the detection of DNA damage and repair.** Methods Mol Biol 1999;113:203-12.

Taningher M, Malacarne D, Perrotta A, Parodi S. **Computer-aided analysis of mutagenicity and cell transformation data for assessing their relationship with carcinogenicity.** Environ Mol Mutagen 1999;33(3):226-39.
Using a computer-aided approach, the tests for Salmonella mutagenicity and transformation in established cell lines were compared for the qualitative bases of their carcinogenicity predictions. For this purpose, a database of 145 chemicals was prepared in which rodent carcinogenicity data and results of the Ames' and transformation tests were available. Using a software program for connectivity analysis (previously developed and validated by us), we assayed the molecular structures of these chemicals for the presence of fragments relatable to their positive (i.e., biophores) or negative (i.e., biophobes) response to the tests in question. These fragments were then studied for their association with genotoxic and nongenotoxic carcinogenicity. The philosophy adopted was that the type and number of molecular fragments chosen by the software to describe the chemicals correctly predicted by the tests could be related to the type of carcinogenic effects to which the tests themselves were sensitive. The classifications made by the software were interpreted by human expertise and the biophores found were compared with the acknowledged structural alerts to DNA reactivity as formalized by Ashby and co-workers [(1991): Mutat Res 257:229-306; (1993): Mutat Res 286: 3-74]. The results show that, in quantitative terms, the overall ability to predict carcinogenicity is about the same for both the Salmonella and transformation tests. However, in qualitative terms the transformation test appears to be sensitive to effects that are more heterogeneous than those inducing mutation, some of which are presumably related to nongenotoxic carcinogenic activities. This study illustrates a possible, innovative model of analysis of chemical structures that, using an automated approach along with the biologist's judgment, could contribute to the detection of complementarities among short-term test endpoints.

Tavan E, Maziere S, Narbonne JF, Cassand P. **Effects of vitamins A and E on methylazoxymethanol-induced mutagenesis in Salmonella typhimurium strain TA100.** Mutat Res 1997 Jul 3;377(2):231-7.
The aim of this study is to report the antimutagenic effect of vitamin A and vitamin E towards methylazoxymethanol (MAM)-induced mutagenesis in Salmonella typhimurium strain TA100 sensitive to alkylating agents. In order to characterize different levels of action of these two fat-soluble vitamins towards the mutagenicity of MAM, several assays have been considered to show the antimutagenic effect and the possible interactions of vitamins with MAM or with the bacteria. Thus, for each vitamin, three different assays with three different incubations have been conducted: (i) MAM, bacteria and vitamins together, (ii) MAM and vitamins, (iii) bacteria and vitamins. The results showed that both vitamins A and E present an antimutagenic effect towards MAM induced mutagenesis. alpha-Tocopherol seems to have an action directly on to the mutagenic agent, whereas the action of retinol is likely due to a protection of the bacterial genoma against MAM. These in vitro results could help to interpret results of colon carcinogenesis studies using animals induced by 1,2-dimethylhydrazine and fed vitamins supplemented diet.

Tsuda S, Kosaka Y, Matsusaka N, Sasaki YF. **Detection of pyrimethamine-induced DNA damage in mouse embryo and maternal organs by the modified alkaline single cell gel electrophoresis assay.** Mutat Res 1998 Jul 8;415(1-2):69-77.
We studied the embryonic and maternal genotoxicity of pyrimethamine (PYR), a potent teratogen and folate antagonist, using alkaline single cell gel electrophoresis (SCG, or Comet) assay as modified by us (we used isolated nuclei instead of isolated cells). ICR mice were treated on the 13th day of pregnancy with a single oral dose of 50 mg PYR/kg. Six maternal organs (liver, kidney, lung, brain, spleen, bone marrow), maternal and fetal placentas, and two embryos were taken 6 and 16 h after treatment; the embryos were divided into head and body portions. Each sample was minced, homogenized gently, and centrifuged. The nuclei from the precipitates were used. PYR induced DNA damage in all maternal organs except spleen and bone marrow 6 h after administration. The DNA damage in all the affected organs was less at 16 h than at 6 h, and that of the kidney and brain returned to control level at 16 h. PYR also induced DNA damage in maternal and fetal placentas and embryos that was detected at 6 and 16 h, with greater damage at 6 h. Co-treatment of folinic acid calcium salt (FNA, 10 mg/kg ip), a reduced active folate form, prevented the PYR-induced DNA damage in all target tissues examined 6 h after treatment. These data indicate that the observed embryonic and maternal DNA damage caused by PYR may be related to folate deficiency, and that the modified alkaline SCG assay can be used to predict fetal/embryonic genotoxicity in vivo, in addition to the organ-specific maternal genotoxicity.

Turnbull D, Frankos VH, Van Delft JH, Devogel N. **Genotoxicity evaluation of wood-derived and vegetable oil-derived stanol esters.** Regul Toxicol Pharmacol 1999;29(2 Pt 1):205-10.
Plant stanol esters from wood and vegetable oil sources were tested for genotoxicity in bacterial (Salmonella typhimurium) and mammalian cell (L5178Y) gene mutation assays and in a mammalian cell chromosome aberration assay (CHO cells). The two stanol ester formulations were tested separately at doses up to the limit of solubility, with and without the addition of an Aroclor-induced rat liver microsome metabolic activation system (S9 mix). All tests were performed in duplicate and gave negative results for both wood and vegetable oil stanol ester formulations. Thus, plant stanol esters are not genotoxic under the conditions of exposure tested.

Turunen MP, Hiltunen MO, Ruponen M, Virkamaki L, Szoka FC Jr, Urtti A, Yla-Herttuala S. **Efficient adventitial gene delivery to rabbit carotid artery with cationic polymer-plasmid complexes.** Gene Ther 1999;6(1):6-11.
Different lipids and cationic polymers were tested in vitro for their ability to transfect rabbit aortic smooth muscle cells and human endothelial cells with lacZ marker gene. Toxicity of the complexes was evaluated with MTT assay. Selected plasmid-polymer complexes with different charge ratios were then tested for in vivo gene transfer efficiency using adventitial gene transfer by placing a silastic gene delivery reservoir (collar) around the carotid artery. Transfection efficiency was determined by X-gal staining 3 days after the gene transfer. Based on in vitro experiments, fractured polyamidoamine dendrimers and polyethylenimines (PEI) were selected for in vivo experiments. Fractured dendrimers (generation 6, +/- charge ratio of 3) had the highest in vivo gene transfer efficiency (4.4% +/- 1.7). PEI with molecular size of 25 kDa (+/- charge ratio 4) was also effective (2.8% +/- 1.8) in this model. PEI of 800 kDa showed a constant but modest gene transfer efficiency (1.8% +/- 0.1) with all charge ratios. A low level gene transfer was also detected with naked DNA (0.5% +/- 0.3). No signs of inflammation

were seen in any of the study groups. We show here that in vitro cell culture experiments can be used to identify efficient in vivo gene transfer methods for arterial gene therapy, but the charge ratios for each complex must be optimized in vivo. It is concluded that fractured dendrimer and PEI are efficient gene delivery vehicles and can be used for arterial gene therapy via adventitial gene delivery route.

Uhl M, Helma C, Knasmuller S. **Single-cell gel electrophoresis assays with human-derived hepatoma (Hep G2) cells.** Mutat Res 1999;441(2):215-24.
The purpose of the present study was the development of a protocol for detecting chemically-induced DNA damage, using the alkaline single-cell gel electrophoresis (SCGE) assay with human-derived, metabolically competent hepatoma (Hep G2) cells. Previous studies indicated that Hep G2 cells have retained the activities of certain phase I and phase II enzymes and reflect the metabolism of genotoxins in mammals better than other in vitro models which require addition of exogenous activation mixtures. The optimal trypsin concentration for the removal of the cells from the plates were found to be 0.1%. Dimethylsulfoxide, at concentrations up to 2%, was an appropriate solvent for water-insoluble compounds. To determine the optimal exposure periods for mutagen treatment, the time kinetics of comet formation was investigated with genotoxic chemicals representing various classes of promutagens namely benzo[a]pyrene (B[a]P), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and N-nitrosodimethylamine (NDMA) and with N-nitrosomethylurea (NMU). All compounds caused a statistically significant induction in DNA damage. With the promutagens, comet formation increased gradually as a function of the exposure duration, and reached maximum values between 20-24 h. With NMU, comet induction maximized already after a short exposure (1 h) and remained at a constant level for up to 24 h. Based on these results, the Hep G2/SCGE assay appears to be a suitable approach for investigating DNA damaging potential of chemicals. Further experiments with IQ and B[a]P showed that the assays are highly reproducible. Comparisons of the present results with those from earlier experiments in which other endpoints (induction of sister chromatid exchanges, micronuclei and chromosomal aberrations) were measured in Hep G2 cells, indicated that the sensitivity of the SCGE assays is more or less identical. Since the SCGE assay is less time consuming than other genotoxicity assays we anticipate that it might be a suitable approach to investigate DNA damaging effects of chemicals in the human-derived, metabolically competent cell line. Copyright 1999 Elsevier Science B. V.

Verschaeve L, Van Gompel J, Thilemans L, Regniers L, Vanparys P, Van Der Lelie D. **VITOTOX bacterial genotoxicity and toxicity test for the rapid screening of chemicals.** Environ Mol Mutagen 1999;33(3):240-8.
The VITOTOX test is a new bacterial genotoxicity test that was previously shown to be very rapid and sensitive. Initially only one Salmonella typhimurium strain (TA104 recN2-4) was used in the test. In this paper we introduce a second strain (TA104pr1) that can be used as an internal control to further enhance the reliability of the test. We demonstrate the usefulness of this pr1 strain in genotoxicity and toxicity testing. We also report on the results of a study where the VITOTOX test was performed on newly synthesized pharmaceutical compounds, or intermediate products in the synthesis of drug candidates. We demonstrate that the test gives identical results when performed independently in two different laboratories and that it correlates well with either the Ames test or SOS chromotest.

Vock EH, Lutz WK, Ilinskaya O, Vamvakas S. **Discrimination between genotoxicity and cytotoxicity**

for the induction of DNA double-strand breaks in cells treated with aldehydes and diepoxides. Mutat Res 1999;441(1):85-93.
The time-dependent dose-response relationships for the induction of DNA double-strand breaks (DSB) assessed by pulsed-field gel electrophoresis (PFGE) and for viability (evaluated by the MTT cytotoxicity test) were investigated in order to discriminate between genotoxic and cytotoxic mechanisms of DNA fragmentation. Cultured human lung epithelial cells (A549) were treated (i) with the aldehydes formaldehyde or glutaraldehyde and (ii) with the DNA-DNA interstrand crosslinkers melphalan, diepoxybutane or diepoxyoctane. Induction of DSB by formaldehyde and glutaraldehyde was seen only after cell viability was reduced to less than about 60% of the control values, indicating that DSB were the consequence of extragenomic damage and viability loss. Melphalan, diepoxybutane and diepoxyoctane induced DSB by a genotoxic mode with concentrations that did not affect cell survival: 8 h after treatment initiation both heat-labile crosslinks and DSB could be detected. Cells were not able to repair the crosslinks induced by diepoxybutane, the crosslinker with the shortest chain length. In contrast, with melphalan and diepoxyoctane, which have a longer crosslinking property considerable repair of crosslinks was observed. The molecular size distribution of the produced DNA fragments supported this mechanistic distinction. The DNA fragments generated by diepoxides were initially large, their concentration decreasing monotonously from 7 Mbp to less than 1 Mbp and were converted to smaller fragments by 72 h in the course of cell death. In contrast, DNA fragments induced by formaldehyde peaked below 1 Mbp, implicating activation of DNA-degrading enzymes. Copyright 1999 Elsevier Science B.V.

Wang JC, Qian BL. **Detection of DNA damage in peripheral lymphocytes by 7 compounds using comet assay.** Chung Kuo Yao Li Hsueh Pao 1997;18(5):451-4.
AIM: To detect the DNA single strand breaks (SSB) in peripheral lymphocytes of mice, rats, and human induced by hydrogen peroxide (H2O2), ethyl methane sulphonate (EMS), dimethylnitrosamine (DMNA), mitomycin C (MMC), benzo (a) pyrene (BaP), cyclophosphamide (CP), and 2-aminofluorene (2-AF). METHODS: Alkaline single cell microgel electrophoresis assay in vitro (comet assay). RESULTS: All were positive with 2 exceptions: EMS (0.97 mmol.L-1) in mice and MMC (30 mumol.L-1) in mice and human. The lowest concentrations detectable were H2O2 (1 mumol.L-1), EMS (0.48 mmol.L-1), BaP (5.0 mumol.L-1), CP (2.0 mmol.L-1), MMC (10 mumol.L-1), DMNA (27.3 mmol.L-1), and 2-AF (62.5 mumol.L-1). CP, BaP, and 2-AF were positive only in the presence of metabolic activation system. CONCLUSION: H2O2, DMNA, BaP, CP, and 2-AF induce SSB in peripheral lymphocytes of mice, rats, and human detected by comet assay, whereas MMC induces SSB only in rats, and EMS in rats and human lymphocytes.

Wang QE, Han CH, Yang YP, Wang HB, Wu WD, Liu SJ, Kohyama N. **Biological effects of man-made mineral fibers (II)--their genetic damages examined by in vitro assay.** Ind Health 1999;37 (3):342-7.
In order to study and compare genetic damage induced by 10 kinds of man-made mineral fibers (JFM fibers) in cells, human lung epithelial cells (A549) were exposed to JFM fibers and chrysotile for 1 h, then single-cell gel electrophoresis (SCGE) assay was used to detect DNA strand breaks, DNA-DNA interstrand crosslink and the ability of DNA to repair; The results showed that all 10 JFM fibers could induce DNA strand breaks, DNA-DNA interstrand crosslinks and inhibit the ability of DNA repair.

When human embryo lung (HEL) cells were exposed to JFM fibers and chrysotile for 24 h respectively, the chromosomal aberration was analyzed and the results showed that chrysotile and most of JFM fibers at 5.0 micrograms/ml induced structural chromosomal aberration, but all of these effects were lower than that of chrysotile and were different among them, suggesting that 10 types of JFM fibers had genotoxicity with different degree in vitro, but all of them were lower than that of chrysotile.

Wessler A. **[Assessment and characterization of genotoxic water contaminants with modern genotoxicological methods]**. Acta Hydrochim Hydrobiol 1999;27(3):164-9. (Ger)
BIOSIS COPYRIGHT: BIOL ABS. The necessity of genotoxicological studies in water requires modern and practicable concepts for a prospective environmental protection. Up to now, genotoxicological studies prefer only the observation and detection of basic genotoxic effects in enriched surface water samples and the fruitless search for the involved water contaminants. In this publication, a new test strategy for the detection and the assessment of genotoxicity in water will be introduced. This strategy allows the detection and l then be better understood. A test strategy in three steps will be proposed. In the first step genotoxicological indicator tests such as comet assay, alkaline filter elution, or the SOS-umu-test are used to get information about the basic status of the tested water samples. In the second step, the positive results of the primary DNA damages will be verified with mutation tests such as the micronucleus assay. The involved biological mechanisms will be biochemically characterized, and the biologi.

Wilson VL, Wei Q, Wade KR, Chisa M, Bailey D, Kanstrup CM, Yin X, Jackson CM, Thompson B, Lee WR. **Needle-in-a-haystack detection and identification of base substitution mutations in human tissues.** Mutat Res 1999;406(2-4):79-100.
Background and induced germline mutagenesis and other genotoxicity studies have been hampered by the lack of a sufficiently sensitive technique for detecting mutations in a small cluster of cells or a single cell in a tissue sample composed of millions of cells. The most frequent type of genetic alteration is intragenic. The vast majority of oncogenic mutations in human and mammalian cancer involves only single base substitutions. We have developed universally applicable techniques that not only provide the necessary sensitivity and specificity for site specific mutagenesis studies, but also identify the point mutation. The exponential amplification procedures of polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been combined with restriction endonuclease (RE) digestion to enable the selective enrichment and detection of single base substitution mutations in human oncogenic loci at a sensitivity of one mutant in more than 10(7) wild type alleles. These PCR/RE/LCR procedures have been successfully designed and used for codons 12 and 248 of the Ha-ras and p53 genes, respectively, both of which contain a natural MspI restriction endonuclease recognition sequence. These procedures have also been adapted for the detection and identification of mutations in oncogenic loci that do not contain a natural restriction endonuclease recognition sequence. Using PCR techniques, a HphI site was incorporated into the codons 12/13 region of the human N-ras gene, which was then used for the selective enrichment of mutants at this oncogenic locus. These PCR/RE/LCR procedures for base substitution mutations in codon 12 of the N-ras gene were found to have the sensitivity of detection of at least one mutant allele in the presence of the DNA equivalent of 10(6) wild type cells. Only one peripheral blood leukocyte DNA specimen out of nine normal individuals displayed an observable Ha-ras mutation that was present at frequency between 10(-5) and 10(-6). These PCR/RE/LCR techniques

for detecting and identifying base substitution mutations are universally applicable to almost any locus or base site within the human or animal genome. With the added advantage of the adjustability of both the amount of DNA (number of genomes) to be tested and the sensitivity (10(-2) to 10(-7)) of the assay selection or enrichment procedures, these PCR/RE/LCR techniques will be useful in addressing a broad range of important questions in mutagenesis and carcinogenesis.

Yoneda A, Asada M, Suzuki M, Imamura T. **Introduction of an N-glycosylation cassette into proteins at random sites: expression of neoglycosylated FGF.** Biotechniques 1999;27(3):576-8, 580, 582 Passim.
We developed a method for introducing an N-glycosylation cassette into proteins at random sites by constructing cDNAs and expressing it in mammalian cells. The protocol entails four steps: (i) generation of cDNAs that contain single, randomly-located blunt end cuts; (ii) ligation of N-glycosylation cassettes into the blunt end cuts in three-frame formats; (iii) selection of the cDNA clones encoding N-glycosylated proteins; and (iv) subcloning into an expression vector for transfection and expression in mammalian cells. This method was evaluated using secreted fibroblast growth factor (FGF) as a model protein. Several secreted FGF cDNA clones, each containing an AsnLeuSer-coding sequence at a random site, were obtained. When these clones were expressed in mammalian cells, some of the secreted FGFs were found to be N-glycosylated. The method described here should also be applicable for random introduction of functional oligopeptide/polypeptide cassettes into virtually any protein of interest.

Zhang Y, Fan W, Kinkema M, Li X, Dong X. **Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene.** Proc Natl Acad Sci U S A 1999;96(11):6523-8.
The Arabidopsis thaliana NPR1 has been shown to be a key regulator of gene expression during the onset of a plant disease-resistance response known as systemic acquired resistance. The npr1 mutant plants fail to respond to systemic acquired resistance-inducing signals such as salicylic acid (SA), or express SA-induced pathogenesis-related (PR) genes. Using NPR1 as bait in a yeast two-hybrid screen, we identified a subclass of transcription factors in the basic leucine zipper protein family (AHBP-1b and TGA6) and showed that they interact specifically in yeast and in vitro with NPR1. Point mutations that abolish the NPR1 function in A. thaliana also impair the interactions between NPR1 and the transcription factors in the yeast two-hybrid assay. Furthermore, a gel mobility shift assay showed that the purified transcription factor protein, AHBP-1b, binds specifically to an SA-responsive promoter element of the A. thaliana PR-1 gene. These data suggest that NPR1 may regulate PR-1 gene expression by interacting with a subclass of basic leucine zipper protein transcription factors.

Zimmer DM, Harbach PR, Mattes WB, Aaron CS. **Comparison of mutant frequencies at the transgenic lambda LacI and cII/cI loci in control and ENU-treated Big Blue mice.** Environ Mol Mutagen 1999;33(3):249-56.
We compared the lambda cII/cI transgenic mutation assay described by Jakubczak et al. [(1996): Proc Natl Acad Sci USA 93:9073-9078] to the previously established Big Blue assay. Genomic DNA isolated from liver, spleen, and lung tissue of control or ethylnitrosourea (ENU)-treated Big Blue mice (100 mg/kg i.p., single dose) was packaged into phage (five animals, two packagings per DNA sample) which were simultaneously plated for lacI and cII/cI mutant frequency (MF) and titer. Mean MF of control animals was higher for cII/cI than lacI for all three tissues examined (spontaneous cII/cI MF divided by

spontaneous lacI MF = 2.9, 3.1, and 1.7 for liver, spleen, and lung, respectively). The differences were statistically significant for liver and spleen, but not lung. The ENU-induced MF measured by subtracting control MFs from ENU-treated MFs was higher in the cII/cI assay than lacI (liver = 23.0 x 10(-5) for cII/cI vs. 15.1 x 10(-5) for lacI; spleen = 64.8 x 10(-5) for cII/cI vs. 36.1 x 10(-5) for lacI; lung = 17.1 x 10(-5) for cII/cI vs. 15.8 x 10(-5) for lacI). Fold increase over control values measured by dividing MF of ENU-treated animals by appropriate control values was higher for lacI than cII/cI (liver = 4.4-fold for lacI vs. 2.7 for cII/cI; spleen = 13.1-fold for lacI vs. 8.4 for cII/cI; and lung = 5.6-fold for lacI vs. 4.0 for cII/cI). Despite these differences, overall results were similar for the two mutational endpoints. These results suggest that the cII/cI assay may be an acceptable alternative to lacI where transgenic mutation studies are indicated.

HEPATIC AND RENAL TOXICITY

Bagdonas S, Dahle J, Kaalhus O, Moan J. **Cooperative inactivation of cells in microcolonies treated with UVA radiation.** Radiat Res 1999;152(2):174-9.
BIOSIS COPYRIGHT: BIOL ABS. Microcolonies of Madison-Darby canine kidney cells (MDCK II) were exposed to UVA radiation, and the number of cells with membrane damage was determined by staining with propidium iodide and fluorescence microscopy. The cells were clearly damaged in a nonrandom manner: The distribution of damaged cells per microcolony was incompatible with the assumption that the cells were damaged independently. The data were accurately described by a so-called propagated damage model in which a damaged cell can.

Barrouillet MP, Potier M, Cambar J. **Cadmium nephrotoxicity assessed in isolated rat glomeruli and cultured mesangial cells: evidence for contraction of glomerular cells.** Exp Nephrol 1999;7(3):251-8.
Cadmium (Cd), an important pollutant, causes severe damage at the renal tubular level. Numerous previous studies have focused upon Cd tubular nephrotoxicity. The present study of Cd-induced glomerular damage examined the vasoactive effect of Cd in freshly isolated glomeruli and mesangial cells. Glomeruli were isolated by passing rat renal cortex pulp through calibrated sieves followed by culture for outgrowth of cells. Quantitative evaluation of glomerular and cellular contractions was performed by morphometric measurement of the area with an automatized image analyzer following different incubation times with Hanks' balanced salt solution or Cd2+. Each glomerulus or mesangial cell served as its own control. Cd lethality was measured with microassay methods (neutral red, MTT uptake, and lactate dehydrogenase release), allowing the determination of an IC50. This ranged from 35 to 60 microM. CdCl2 induced a time-dependent contractile effect on isolated glomeruli; planar surface area decreases were 6.9% (1 microM), 7.5% (0.1 microM), and 7% (0.01 microM). The decrease started as soon as Cd was in contact with glomeruli and ended 40 min later: T5 (2%), T10 (3.5%), T20 (4. 2%), T30 (6.3%), T40 (7%). Cell size reduction was 19% (1 microM), 14% (0.1 microM), and 18% (0.01 microM) and was also time-dependent. To confirm that contractile events occurred during the cell shape changes, examination of the mesangial alpha-actin network was performed concurrently. These results indicate that Cd contracts glomerular structures. This may, in part, explain the reduction in glomerular filtration seen in Cd nephrotoxicity.

Boon PJ, Marinho HS, Oosting R, Mulder GJ. **Glutathione conjugation of 4-hydroxy-trans-2,3-**

nonenal in the rat in vivo, the isolated perfused liver and erythrocytes. Toxicol Appl Pharmacol 1999;159(3):214-23.
The formation of glutathione (GSH) conjugates of racemic 4-hydroxy-trans-2,3-nonenal (4-HNE) in the rat in vivo in the perfused rat liver and rat erythrocytes has been studied. An HPLC system was developed for the assay of 4-HNE-glutathione conjugates (HNE-SG). The very sensitive electrochemical detection method (detection limit 5 pmol) can also be used to study endogenously formed HNE-SG. Three diastereomeric HNE-SG conjugates could be separated by this system. Rat liver cytosol catalyzed the formation of 2 of the 3 conjugates. When 17 &mgr;mol/kg [(3)H] 4-HNE was injected intravenously in the rat, 21% of the radioactivity was excreted within 90 min in bile and 37% in urine. Most of the 4-HNE in bile was present as 2 of the HNE-SG conjugates (molecular mass 463). In addition, 25% was excreted as a third GSH conjugate (molecular mass of 461), which was identified as the lactone of the 4-hydroxynonenoic acid glutathione conjugate. Erythrocytes in vitro eliminated 4-HNE very rapidly, in part by GSH conjugation, suggesting that they may also play an important role in vivo. To study the role of the liver selectively, we used the recirculating perfused rat liver without erythrocytes in the perfusion medium; the same conjugates were found, but the third conjugate was a minor component. These results present direct evidence for the in vivo formation of 4-HNE glutathione conjugates in which the liver may play an important role. Copyright 1999 Academic Press.

Braunbeck T, Appelbaum S. **Ultrastructural alterations in the liver and intestine of carp Cyprinus carpio induced orally by ultra-low doses of endosulfan.** Dis Aquat Organ 1999;36(3):183-200.
In order to elucidate the importance of food-borne chemical contamination in fish, cytological and ultrastructural alterations in hepatocytes and enterocytes of common carp Cyprinus carpio L. exposed for 5 wk to 0.5 microgram endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9a-hexahydro-6,9-methano-2,4,3- -benzo- dioxyanthiepin-3-oxide) kg-1 food dry weight, equivalent to an ultra-low dosis of 15 ng kg-1 fish d-1, were investigated by means of light and electron microscopy. Observations on liver alterations were quantified by morphometric analysis. Livers show enlargement of the nucleolus, increase in number and size of both Golgi fields and rough endoplasmic reticulum (ER) lamellae, as well as proliferation of peroxisomes and lysosomes. Taken together, these alterations represent the morphological equivalent of a general stimulation of hepatic metabolism. Proliferation of the smooth ER is indicative of the onset of biotransformation processes under the influence of food-borne endosulfan. Further pathological processes in the liver were evident by glycogen and lipid depletion, invasion of phagocytic macrophages, and accumulation of myelinated bodies in endothelial cells of hepatic sinusoids. In the intestinal tract, exposure to endosulfan is associated with a complete lack of chylomicrons in the epithelial lining, which indicates disturbance of intestinal absorption. The reaction of the gut epithelium also included considerable distension of the intercellular space and an elevated number of lysosomal inclusions in enterocytes. An increased rate of mucous cell precursors was detectable, and macrophages were numerous. Results are consistent with endosulfan resorption by the intestinal epithelium and the coexistence of gut and liver ultrastructural changes at extremely low doses. Thus, the substantiation of pathological alterations in organs sequentially in contact with toxicants appears useful as a biomarker of pollutant exposure and effect. With regard to a chemical spill into the Rhine river at Basel, Switzerland, in November 1986, endosulfan, as a component of the mixture of toxic substances, may well have contributed to the overall toxicity of the chemicals released during the accident and the subsequent fish kill, less as a toxicant in itself than as a stimulant for the toxicity of

other xenobiotics.

Chatterjee PK, Cuzzocrea S, Thiemermann C. **Inhibitors of poly (ADP-ribose) synthetase protect rat proximal tubular cells against oxidant stress.** Kidney Int 1999;56(3):973-84.
BACKGROUND: The generation of reactive oxygen species (ROS) has been implicated in the pathogenesis of renal ischemia-reperfusion injury. ROS produce DNA strand breaks that lead to the activation of the DNA-repair enzyme poly (ADP-ribose) synthetase (PARS). Excessive PARS activation results in the depletion of its substrate, nicotinamide adenine dinucleotide (NAD) and subsequently of adenosine 5'-triphosphate (ATP), leading to cellular dysfunction and eventual cell death. The aim of this study was to investigate the effect of various PARS inhibitors on the cellular injury and death of rat renal proximal tubular (PT) cells exposed to hydrogen peroxide (H2O2). METHODS: Rat PT cell cultures were incubated with H2O2 (1 mM) either in the presence or absence of the PARS inhibitors 3-aminobenzamide (3-AB, 3 mM), 1,5-dihydroxyisoquinoline (0.3 mM) or nicotinamide (Nic, 3 mM), or increasing concentrations of desferrioxamine (0.03 to 3 mM) or catalase (0.03 to 3 U/ml). Cellular injury and death were determined using the MTT and lactate dehydrogenase (LDH) assays, respectively. H2O2-mediated PARS activation in rat PT cells and the effects of PARS inhibitors on PARS activity were determined by measurement of the incorporation of [3H]NAD into nuclear proteins. RESULTS: Incubation of rat PT cells with H2O2 significantly inhibited mitochondrial respiration and increased LDH release, respectively. Both desferrioxamine and catalase reduced H2O2-mediated cellular injury and death. All three PARS inhibitors significantly attenuated the H2O2-mediated decrease in mitochondrial respiration and the increase in LDH release. Incubation with H2O2 produced a significant increase in PARS activity that was significantly reduced by all PARS inhibitors. 3-Aminobenzoic acid (3 mM) and nicotinic acid (3 mM), structural analogs of 3-AB and Nic, respectively, which did not inhibit PARS activity, did not reduce the H2O2-mediated injury and necrosis in cultures of rat PT cells. CONCLUSION: We propose that PARS activation contributes to ROS-mediated injury of rat PT cells and, therefore, to the cellular injury and cell death associated with conditions of oxidant stress in the kidney.

Chen J, Kunos G, Gao B. **Ethanol rapidly inhibits IL-6-activated STAT3 and C/EBP mRNA expression in freshly isolated rat hepatocytes.** FEBS Lett 1999;457(1):162-8.
The ability of ethanol to inhibit regenerative processes in the liver is thought to play a key role in the development of alcoholic liver disease. To understand the underlying mechanisms, we investigated the effects of ethanol on the Janus kinasesignal transducer and activator transcription factor (JAK-STAT) signaling pathways in hepatocytes. Treatment of freshly isolated adult rat hepatocytes with 10-100 mM ethanol rapidly (< 3 min) inhibits interleukin-6 (IL-6)-induced STAT3 activation, tyrosine and serine phosphorylation and IL-6-induced CCAAT enhancer binding protein (C/EBP) alpha and beta mRNA expression. Western analyses, in vitro kinase assays and in vivo cell labelling assays indicate that this inhibitory effect is not due to blocking the upstream-located JAK1, JAK2 or Tyk2 activation. On the contrary, acute ethanol exposure significantly potentiates IL-6-induced JAK1 autophosphorylation in vitro and in vivo. Pretreatment with sodium vanadate, a non-selective tyrosine phosphatase inhibitor, or with MG132 and lactacystin, proteasome inhibitors, does not abolish the ethanol inhibition of IL-6-induced STAT3 activation, suggesting that activation of protein tyrosine phosphatases or the ubiquitin-proteasome pathway is not involved. In view of the critical role of IL-6 signaling in liver regeneration,

these findings suggest that the ability of biologically relevant concentrations of ethanol to markedly inhibit IL-6-induced STAT3 phosphorylation is one of the cellular mechanisms involved in the pathogenesis and progression of alcoholic liver diseases.

Chien M, Astumian M, Liebowitz D, Rinker-Schaeffer C, Stadler WM. **In vitro evaluation of flavopiridol, a novel cell cycle inhibitor, in bladder cancer.** Cancer Chemother Pharmacol 1999;44 (1):81-7.
PURPOSE: To determine the in vitro effects of flavopiridol on bladder cancer cell lines, immortalized urothelial cell lines, and normal urothelial cells well characterized for defects in p53, pRb, and p16. METHODS: Growth inhibition was assessed via an MTT assay and apoptosis via DAPI nuclear staining. Cell cycle analysis was performed via propidium iodide staining and fluorescent activated cell sorting (FACS). Multidrug-resistant cells were generated by continuous exposure to doxorubicin. RESULTS: Growth inhibition was not correlated with inactivation of p53, pRb, or p16. All cells experienced G2/M arrest within 24 h of flavopiridol exposure. Modest apoptosis was observed but required 72 h of continuous drug exposure to become evident. There was no obvious synergistic or antagonistic toxicity when flavopiridol was combined with radiotherapy or cisplatin dosed at the IC50 despite the observation that radiotherapy and flavopiridol led to more profound G2/M arrest than either agent alone. Doxorubicin-resistant cells, demonstrated to overexpress the MDR1 multi-drug-resistance protein were equally as sensitive to flavopiridol as the parental cells. CONCLUSIONS: Flavopiridol is a novel cell cycle inhibitor that may be a useful agent in bladder cancers with tumor suppressor gene alterations and/or multidrug resistance.

Dartsch PC, Hildenbrand S, Kimmel R, Schmahl FW. **Investigations on the nephrotoxicity and hepatotoxicity of trivalent and hexavalent chromium compounds.** Int Arch Occup Environ Health 1998 Sep;71 Suppl:S40-5.
In contrast to trivalent chromium (Cr(III)) compounds, hexavalent chromium ((Cr(VI)) compounds are oxidizing agents capable of directly inducing tissue damage and possessing carcinogenic, mutagenic and teratogenic potency. After oral or dermal absorption of Cr(VI), the kidney is the main target organ for chromium accumulation, which might result in acute tubular necrosis in humans. In contrast, an acute toxic effect of Cr(VI) on the liver has not yet been described. Therefore, we used two established epithelial cell lines from the kidney (Opossum kidney cells) and the liver (Hep G2 cells) to design an in vitro-assay which is able to examine acute toxic effects of chromium compounds. Cells of both cell lines were treated with various concentrations of Cr(III) and Cr(VI) ranging from 0.01 micromol/l to 1 mmol/l for 24 h. Thereafter, cell morphology, organization of the intracellular cytoskeleton, number of viable cells and mean cell volume were examined. The results show that Cr(VI), but not Cr(III), has an acute cytotoxic effect and causes a dose-dependent loss in cell viability. The effective dose that caused 50% of cell death was 5 micromol/l for kidney epithelial cells and 50 micromol/l for liver epithelial cells. This means that kidney epithelial cells are 10 times more sensitive towards Cr(VI) treatment than liver epithelial cells and this might explain the known nephrotoxicity in vivo. The loss in cell viability was accompanied by a rounding and detachment of the cells and a marked reduction of intracellular F-actin-containing stress fibers. Microtubules and intermediate-sized filaments were observed to be unaffected. Only in the case of kidney epithelial cells, a dose-dependent cell volume increase was observed after Cr (VI) treatment at concentrations up to 50 micromol/l. At higher concentrations, the cell volume

decreased due to the high number of cells undergoing lysis and the appearance of cellular fragments. Various chloride channel blockers with different specificities, molecular structures and inhibitory potentials were tested for their ability to prevent Cr(VI)-induced cell damage. None of the channel blockers was able to inhibit cell damage, suggesting that the uptake of Cr(VI) through the general anion transport system of the cell membrane might be only one facet of cellular uptake and toxification. The data presented here not only confirm the different organ-specific effects of Cr(III) and Cr(VI), but also provide a basis for future experiments on the understanding of acute toxicity of Cr(VI) compounds. Moreover, the results demonstrate that the designed in vitro-assay might be a useful tool to prove whether non-toxic Cr(III) can be oxidized to Cr(VI) under specific industrial conditions (for example, in the leather or chrome industry).

Finn WF, Porter GA. **Urinary biomarkers: recommendations of the Joint European/United States Workshop for future research.** Ren Fail 1999;21(3-4):445-51.
The session concluded on a positive note with enthusiasm on the part of participates to become involved in one of the proposed joint protocols. Left to be answered was whether or not individual urinary biomarkers can be tailored to be disease specific or will there always be a need for a panel of biomarkers to insure interpretable results? It was agreed that proposals for three studies would be prepared. The first study will take advantage of the fact that field studies are currently being organized by both European and American groups. European scientists are working with the World Health Organization to investigate lead exposure in a region of China. At the same time, scientists in the United States are implementing a surveillance program in a population exposed to lead and other heavy metals in Kellogg, Idaho. These efforts provide an excellent opportunity for the sharing of samples and the study of a biomarkers panel that would contain both standard and candidate biomarkers. It was agreed that the parties interested in participating would alert the workshop organizers. The second study will expand upon a protocol developed by Dr. Debroe that has as its subjects non-transplant patients being treated with cyclosporine. An additional complimentary study of tacrolimus (FK-506) nephrotoxicity will also be developed. This protocol will be designed to follow the loss of renal function with a urinary biomarkers panel. The third study will follow the lead of Dr. Safirstein who urged the consideration of Cisplatin nephrotoxicity as a singular model for analyzing the usefulness of various biomarkers as measures of both acute and chronic nephrotoxicity.

Galigniana MD, Piwien-Pilipuk G. **Comparative inhibition by hard and soft metal ions of steroid-binding capacity of renal mineralocorticoid receptor cross-linked to the 90-kDa heat-shock protein heterocomplex.** Biochem J 1999;341(Pt 3):585-92.
We analysed the inhibitory effects in vitro and in vivo of several metal ions on aldosterone binding to the rat kidney mineralocorticoid receptor with the purpose of assessing possible toxic effects of those ions on sodium retention, as well as to obtain information on receptor structural requirements for ligand binding. For the assays in vitro, the inhibitory effects of 20 metal ions were analysed on steroid-binding capacity for renal receptor cross-linked to 90-kDa heat-shock protein (hsp90) by pretreatment with dimethyl pimelimidate. Cross-linking prevented the artifactual dissociation of hsp90 (and, consequently, the loss of steroid binding) from the mineralocorticoid receptor due to the presence of high concentrations of salt in the incubation medium. Cross-linked heterocomplex showed no difference in ligand specificity and affinity with respect to native receptor, but increased stability upon thermal- or

ionic-strength-induced destabilization was observed. Treatments in vitro with metal ions in the range 10(-8)-10(-1) M resulted in a differential inhibitory effect for each particular ion on aldosterone binding. Using the negative logarithm of metal concentration for 50% inhibition, the ions could be correlated with their Klopman hardness constants. The analysis of this relationship led us to postulate three types of reaction: with thiol, imidazole and carboxyl groups. The essential role played by these residues in steroid binding was confirmed by chemical modification of cysteines with dithionitrobenzoic acid, histidines with diethyl pyrocarbonate and acidic amino acids with Woodward's reagent (N-ethyl-5-phenylisoxazolium-3'-sulphonate). Importantly, the toxic effects of some metal ions were also observed by treatments in vivo of adrenalectomized rats on both steroid-binding capacity and aldosterone-dependent sodium-retaining properties. We suggest that those amino acid residues are involved in the activation process of the mineralocorticoid receptor upon steroid binding. Thus toxic effects observed with these metal ions may be a consequence of modifications of those essential groups. Our results support the notion that toxicity of metals on renal mineralocorticoid function may be predicted according to their chemical hardness.

Grandaliano G, Ranieri E, Monno R, Gesualdo L, Schena F. **Ramipril inhibits in vitro human mesangial cell proliferation and platelet-derived growth factor expression.** Exp Nephrol 1999;7 (3):229-35.
Angiotensin-converting enzyme (ACE) inhibitors are antihypertensive drugs that have been shown to reduce proteinuria and to slow down the progression of renal function deterioration in different models of chronic glomerular disease. Major pathogenetic features of progressive glomerular injury leading to glomerulosclerosis are mesangial cell proliferation and platelet-derived growth factor (PDGF) expression. The aim of the present study was to evaluate the effect of ramipril, an ACE inhibitor, on these two potential therapeutic targets. Thus, the effect of ramipril on DNA synthesis, cell proliferation and PDGF A and B chain gene expression in fetal calf serum (FCS)-activated cultured human glomerular mesangial cells was investigated. DNA synthesis was evaluated by tritiated thymidine incorporation, cell proliferation by direct cell counting and cell viability by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). PDGF A and B chain gene expressions were studied by Northern blot and RT-PCR, respectively. In a dose-dependent manner ramipril inhibited the FCS-induced DNA synthesis and cell proliferation. This effect was not dependent upon a toxic effect as demonstrated by MTT. The antiproliferative effect of ramipril was most likely independent of its ability to inhibit ACE present in the FCS and/or expressed by the cells, since a synthetic peptide that specifically inhibits ACE, at the same molar concentrations, did not inhibit FCS-stimulated DNA synthesis. Moreover, ramipril significantly reduced FCS-induced PDGF A and B chain gene expression. Finally, ramipril completely abolished the PDGF A and B chain gene expression induced by phorbol 12-myristate 13-acetate, a specific protein kinase C activator, suggesting a site of action downstream of this enzyme in the mitogenic signal transduction pathway. Our study would suggest that the modulatory action of ramipril on activated mesangial cell proliferation and PDGF expression is independent of its ability to inhibit ACE and could represent an additional mechanism in the renal protective effects of this drug.

Komatsuda A, Wakui H, Oyama Y, Imai H, Miura AB, Itoh H, Tashima Y. **Overexpression of the human 72 kDa heat shock protein in renal tubular cells confers resistance against oxidative injury**

and cisplatin toxicity. Nephrol Dial Transplant 1999;14(6):1385-90.
BACKGROUND: Recent studies have shown that the 72-kDa heat shock protein (HSP72) can be induced in renal tubular cells by a variety of stress conditions, and suggested its cytoprotective function. We have tested this hypothesis directly by transfection studies. METHODS: LLC-PK1 cells (porcine renal tubular epithelial cells) were stably transfected with pBK-CMV or pBK-CMV containing the human HSP72 gene (pBK-CMV-HSP72). These cells were then treated with various concentrations of hydrogen peroxide or cisplatin. The cell viability and lytic cell damage were determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and lactate dehydrogenase release assay. RESULTS: Immunoblot and immunocytochemical analyses showed the high level expression of HSP72 in LLC-PK1 cells transfected with pBK-CMV-HSP72. In addition, the expression of other major HSPs (HSP90, HSP73, HSP60 and HSP27) was not affected by transfection. LLC-PK1 cells overexpressing HSP72 were significantly more resistant to hydrogen peroxide and cisplatin treatments than control cells. CONCLUSION: These results indicate that overexpressed HSP72 plays a direct role in protecting renal tubular cells against oxidative injury and cisplatin toxicity.

Li HP, Geng L, Burrow CR, Wilson PD. **Identification of phosphorylation sites in the PKD1-encoded protein C-terminal domain.** Biochem Biophys Res Commun 1999;259(2):356-63.
The PKD1-encoded protein, "polycystin-1", has a large N-terminal extracellular portion, multiple transmembrane domains, and a short intracellular C-terminal tail with four tyrosine residues and two putative sites for serine phosphorylation. Its function in kidney development and autosomal dominant polycystic kidney disease (ADPKD) is still unknown. We have subcloned the cDNA encoding the polycystin-1 C-terminal domain (PKD1-CTD) into a prokaryotic expression vector, and site-directed mutagenesis was performed to target the four tyrosine residues and four serine residues in two putative phosphorylation sites. In vitro phosphorylation assays were conducted on both wild type and mutant PKD1-CTD fusion proteins. It was found that the wild type PKD1-CTD and all mutant fusion proteins, except S4251G/S4252G, could be phosphorylated by lysates from cultured normal human renal collecting tubule (NHCT) cells, as well as by commercially purified cAMP-dependent protein kinase (PKA). The phosphorylation of the PKD1-CTD fusion protein by NHCT lysates was greatly enhanced by cAMP and its analog 8-Br-cAMP, and inhibited by the specific PKA inhibitors PKI(6-22) and H-89. Activators and inhibitors of protein kinase C (PKC) had no effects on the phosphorylation of the PKD1-CTD fusion protein. Using commercially purified pp60(c-src) (c-src) it was also shown that the PKD1-CTD fusion protein could be phosphorylated by c-src in vitro, and that this phosphorylation could be abolished by a mutation Y4237F. By comparing the amino acid sequence at 4249-4253 (RRSSR) with the consensus sequence for PKA phosphorylation (RRXSX), we suggest that the serine residue at 4252 is the target of phosphorylation by a cAMP-dependent protein kinase in NHCT cell lysates. In addition, we suggest that Y4237 might be phosphorylated by c-src in living cells. Copyright 1999 Academic Press.

Liang X, Qiao Z, Yin L. **[Lipopolysaccharide-induced apoptosis of rat hepatocyte in vitro]**. Chung Hua Kan Tsang Ping Tsa Chih 1999;7(2):72-3. (Chi)
OBJECTIVES: To investigate the effect of lipopolysaccharide(LPS) on hepatocyte in vitro. METHODS: The hepatocytes were isolated in the way of liver perfusion with 0.05% collagenase type I and type IV, and cultured for 24 h in vitro before LPS was added directly into the culturing medium. Propidium iodide (PI) staining, and transmission electron microscopy techniques had been used to observe the

morphological changes of hepatocyte treated with LPS. DNA-fragment assay was analyzed by the agarose gel electrophoresis to determine apoptotic level. RESULTS: Hepatocytes incubated with LPS exhibited some typical apoptosis-specific morphological features. The DNA-fragment by agarose gel electrophoresis demonstrated the typical ladder pattern on the hepatocytes directly exposed to LPS, but it was absent in the group used ATA, an inhibitor of apoptosis. These morphological changes, accompanied by DNA fragmentation assay, confirmed that cells were dying through an apoptotic pathway. In addition, the hepatocyte number of apoptosis increased parallel with the dose of LPS and time within 24 h when hepatocytes were exposed to LPS alone. CONCLUSIONS: LPS can induce apoptosis of hepatocyte in vitro.

Paradis V, Dargere D, Vidaud M, De Gouville AC, Huet S, Martinez V, Gauthier JM, Ba N, Sobesky R, Ratziu V, et al. **Expression of connective tissue growth factor in experimental rat and human liver fibrosis.** Hepatology 1999;30(4):968-76.
Connective tissue growth factor (CTGF) stimulates in vitro fibroblast proliferation and extracellular matrix synthesis. The aim of this study was to assess the role of CTGF in liver fibrogenesis. CTGF expression was investigated both at the protein and mRNA level in biopsies of chronic liver diseases, in experimental models of liver fibrosis, and in hepatic stellate cells in culture. CTGF immunostaining was observed in most human liver biopsies with significant fibrosis. An increase of CTGF immunostaining was associated with a higher score of fibrosis both in the group of chronic hepatitis C (chi(2) = 9.3; P <.01) and in the non-hepatitis C group (chi(2) = 7.2; P <.02). In situ hybridization showed CTGF mRNA expression in spindle cells in both the fibrous septa and sinusoidal lining. In experimental models of liver fibrosis, CTGF accumulated in parallel with the development of septal fibrosis and cirrhosis. Quantification of CTGF mRNA by a real-time reverse-transcription polymerase chain reaction (RT-PCR) assay showed a significant increase of CTGF mRNA in both CCl(4)-induced and bile duct-ligated rat models of liver fibrosis. Expression of CTGF protein and mRNA was definitively assigned to hepatic stellate cells, because CTGF was detected by Western blot both in lysate and supernatant of a hepatic stellate cell line derived from rats. These cells also displayed CTGF protein and mRNA as shown by immunohistochemistry and in situ hybridization. In conclusion, this study shows that CTGF is strongly expressed during liver fibrogenesis, and hepatic stellate cells seem to be the major cellular sources of CTGF in the liver.

Pariente JL, Bordenave L, Bareille R, Ohayon-Courtes C, Baquey C, Le Guillou M. **In vitro cytocompatibility of radio-opacifiers used in ureteral endoprosthesis.** Biomaterials 1999;20(6):523-7.
Ureteral endoprostheses are urinary catheters made of polymeric biomaterials made radio-opaque through the addition of X-ray absorbing additives such as barium, bismuth, tantale or tungsten. The aim of this work was to study the in vitro toxicity of solutions of these radio-opacifiers using two cell culture models. Primary-cultures of human urothelial cells (HUC) arising from normal adult urinary tract and permanent urothelial cell line were used. Solutions at different dilutions were placed into the wells containing monolayers of confluent cells. After 24 h incubation period, the solutions were removed and cell viability and cell metabolic activity tests were performed (Neutral Red assay and MTT assay). At a concentration lower than 1 mg l(-1) the different radio-opacifiers used showed no toxicity. From 1 to 3 mg l(-1) one can note a significant dose-dependent decrease of cell metabolic activity of solely HUC for

barium chloride. At 3 mg l(-1) one can note a significant deleterious effect on HUC metabolic activity, with bismuth and tantale. For tungsten, there is no deleterious effect, but on the contrary a significant increase in HUC metabolic activity at a 0.5 mg l(-1) concentration. None of the solutions did provoke alterations in HUC viability for concentrations less than 3 mg l(-1). Interestingly, for permanent cell line one can note a solely significant decrease of cell viability at 3 mg l(-1) for tantale. All the other tested salts on permanent cell line were not significantly different from controls for cell viability as well as cell metabolic activity. HUC culture model may be of relevance for the screening of radio-opacifiers intended for ureteral endoprostheses.

Prakash S, Nanji AA, Robbins PW. **Fibrosin: a novel lymphokine in alcohol-induced fibrosis.** Exp Mol Pathol 1999;67(1):40-9.
In this study, we examined the role of fibrogenic cytokines in alcohol-induced fibrosis. In particular, we examined the production of a novel fibrogenic cytokine, fibrosin, among others, by fibroblasts in response to ethanol in vitro; we also studied the production of fibrosin in an animal model of alcohol-induced liver injury. This model system utilizes the intragastric feeding rat model in which rats are fed different dietary fats and ethanol or dextrose. Our study showed that physiologic concentrations of ethanol directly induced proliferation of fibroblasts in vitro and also stimulated the production of cytokines. In particular, fibrosin, the novel fibrogenic cytokine, was produced. Other cytokines such as TGFbeta, IL-6, and TNFalpha were also induced. Also, exposure of fibroblasts to interleukin-1beta, interleukin-6, and tumor necrosis factor alpha induced production of fibrosin. In the fish oil-ethanol-fed rats which showed fibrotic lesions in the liver, fibrosin mRNA as well as protein was expressed. Fibrosin was not detected in control rats not exhibiting fibrosis. These studies show that ethanol can directly stimulate fibroblast proliferation and production of fibrogenic cytokines. It is likely that fibrosin, which may be derived from inflammatory cells, contributes to alcohol-induced hepatic fibrosis in vivo.

Saso L, Valentini G, Leone MG, Grippa E, Silvestrini B. **Development of an in vitro assay for the screening of substances capable of dissolving calcium oxalate crystals.** Urol Int 1998;61(4):210-4.
Despite the risk of kidney damage, lithotripsy is the usual way of treating calcium oxalate (CaOx) stones, the most common type of nephrolithiasis, because no effective chemolytic agents are available. However, the search of new calcium chelators, less toxic than the current ones, continues, and some of them could be tested in experimental models of nephrolithiasis, after their ability of dissolving CaOx crystals is verified. In this connection, we developed a simple assay that requires only inexpensive equipment available in most laboratories for the screening of substances potentially capable of dissolving CaOx crystals. In particular, we decided to investigate whether substances previously shown to inhibit CaOx precipitation were also capable of dissolving this salt. Briefly, CaOx tablets of highly reproducible weight (4.55 +/- 0.07 mg) were prepared by spinning, at high speed (16,000 g), microcentrifuge tubes in which 500 microl aliquots of 0.1 M sodium oxalate and 0.1 M calcium chloride at pH 6 were added. When these tablets were incubated overnight with solutions at different concentrations of EDTA, sodium citrate, manganese chloride, sodium sulfate, sodium chloride, malic acid, succinic acid and gluconic acid, a significant dissolving activity was observed for EDTA (approximately 25% at 0.25 M), sodium citrate (approximately 30% at 1 M) and manganese chloride (approximately 20% at 0.5 M). A good linear correlation ($r2 = 0.84$, $p < 0.05$) was found between the

affinity for calcium and the activity of EDTA, sodium citrate, sodium sulfate, malic acid, succinic acid and gluconic acid, indicating that these compounds act mainly by chelating the calcium ion. Instead, manganese was supposed to act by interacting with the oxalate ion.

Scholz S, Segner H. **Induction of CYP1A in primary cultures of rainbow trout (Oncorhynchus mykiss) liver cells: concentration-response relationships of four model substances.** Ecotoxicol Environ Saf 1999;43(3):252-60.
The study aims to evaluate short-term teleost hepatocyte cultures to establish dose-response curves for CYP1A induction and to rank the relative potencies of xenobiotics. Hepatocytes isolated from rainbow trout (Oncorhynchus mykiss) were incubated in vitro in coculture with RTG-2 cells in serum-free, chemically defined medium. Concentration-dependent induction of cytochrome P4501A was observed after treatment of the hepatocytes for 48 h with one of the four polyaromatic hydrocarbons: 3-methylcholanthrene, benzo[a]pyrene, benz[a]anthracene, and beta-naphthoflavone. The induction response was assessed by measuring 7-ethoxyresorufin-O-deethylase (EROD) and 7-ethoxycoumarin-O-deethylase (ECOD). From the data, EC50 and maximal induction response values were calculated. The rank order of EC50 values differed among the four model compounds, depending on the endpoint parameter. EC50 values for EROD and ECOD also differed in comparison to published affinity data for mammalian arylhydrocarbon receptor. The results of this study indicate the potential of primary teleostean hepatocyte cultures for studies on induction potency and regulation of piscine cytochrome P4501A. A drawback, however, appears to be the pronounced interindividual variation of the quantitative response of the cells. Copyright 1999 Academic Press.

Skett P, Roberts P, Khan S. **Maintenance of steroid metabolism and hormone responsiveness in cryopreserved dog, monkey and human hepatocytes.** Chem Biol Interact 1999;121(1):65-76.
The efficient and effective use of hepatocytes from larger species and rare human material requires a reliable storage method for cells not needed on the day of preparation. Cryopreservation would seem to be the only viable alternative. In this study the suitability of a published cryopreservation technique on dog, monkey and human hepatocytes has been examined and the cells were tested for functionality directly after thawing and subsequent to culture using steroid metabolism and hormone responsiveness of glycogen phosphorylase a. Monkey and human hepatocytes appear to survive the freezing and thawing process better than dog cells-the latter losing the ability to respond to adrenergic stimuli and their ability to maintain steroid metabolism in culture. Although monkey and human cells do preserve their steroid metabolising capacity after freeze/thawing, there is not the significant increase in enzyme activity seen during culturing freshly isolated cells. It would appear, therefore, that some damage has occurred to the cells during the freeze/thaw process. As previously noted, Williams' medium E is superior to Ham's F-10 in maintaining enzyme activities in culture. It is suggested that cryopreservation is the way forward for the development of stockpiles of viable hepatocytes for biomedical and toxicological research and development but that further modifications to the process are still necessary to optimise the maintenance of liver-specific functions in the thawed cells.

Slaughter MR, Bugelski PJ, O'Brien PJ. **Evaluation of Alamar Blue reduction for the in vitro assay of hepatocyte toxicity.** Toxicol In Vitro 1999;13(4-5):567-9.
BIOSIS COPYRIGHT: BIOL ABS. Alamar Blue (AB) reduction is a promising new in vitro assay which is simple to conduct and amenable to repeated measurements and high-throughput screening;

however, evaluation with hepatocytes has not been reported. Accordingly, we compared AB reduction with established markers of hepatocyte viability and cell density. Primary rat hepatocytes were allowed to adhere to collagen-coated 96-well plates, then exposed for 16 hours to culture medium, 0.7% dimethyl sulfoxide (DMSO) in medium, 500 muM C nd good correlation with NR uptake, LDH release, TP and cell density. AB assay precision variedwith cell density, but was similar to other assays in cytotoxicity screening. Good correlation with cell density indicates AB to have the potential for assessment of hepatocyte proliferation. From the results reported here, we recommend further evaluation and optimization of a protocol for application of AB reduction as a test for cytotoxicity and proliferation in primary hepatocyte cultures.

Tamatani T, Hattori K, Iyer A, Tamatani K, Oyasu R. **Hepatocyte growth factor is an invasion/ migration factor of rat urothelial carcinoma cells in vitro.** Carcinogenesis 1999;20(6):957-62.
Hepatocyte growth factor (HGF) plays an important role in the growth, progression and angiogenesis of various tumors. It is reported that patients with urinary bladder cancer have elevated levels of HGF in urine and that bladder cancer tissue contains an increased amount of HGF. Thus, the data suggest a functional role of HGF in urinary bladder cancer. We evaluated the mechanistic role of HGF in urinary bladder carcinoma in vitro using the rat urothelial cell lines MYP3 (anchorage-dependent and non-tumorigenic in athymic nude mice), LMC19, MYU3L, T6 and AS-HTB1 (anchorage-independent and tumorigenic). The HGF receptor c-met was expressed by all of the cell lines, as determined by northern blot. In MYP3 cells, HGF strongly stimulated anchorage-dependent growth, but not migration, invasion or secretion of matrix metalloproteinases (MMPs). In LMC19, T6 and AS-HTB1 cells, HGF stimulated migration, invasion and secretion of MMPs. Anchorage-dependent growth stimulation was limited to AS-HTB1 cells. MYU3L cells were refractory to HGF in both growth and invasion assays. However, a neutralizing antibody and an anti-sense oligonucleotide to HGF partially inhibited the growth only of MYU3L cells, the finding being indicative of an autocrine stimulatory mechanism. HGF mRNA expression and protein synthesis were induced in bladder stromal cells by the conditioned medium of carcinoma cell lines, and IL-1beta and basic fibroblast growth factor were identified as cancer cell-derived HGF-releasing factors. These results suggest that HGF acts as a mitogen in a non-tumorigenic cell line, whereas in tumorigenic cell lines it acts as an invasion and migration factor by either a paracrine or an autocrine mechanism.

Whiting PH, Tisocki K, Hawksworth GM. **Human renal medullary interstitial cells and analgesic nephropathy.** Ren Fail 1999;21(3-4):387-92.
The aim of this study was to investigate the effects of known papillotoxins using cultures of human renal interstital medullary cells (hRMIC). The culture of hMIC was based on the primary culture of human renal medullary explants, selective detachment of interstitial cells and selective overgrowth of these cells in a serum-rich medium after dilution cloning. The homogeneous population of cells obtained exhibited the characteristic morphological and functional characteristics of Type I interstitial cells, viz. stellate-shaped cells demonstrating numerous lipid droplets, abundant endoplasmic reticulum and mitochondria, fine filaments underlying the cell membrane and the production of extracellular matrix. Cytotoxicity studies using hMIC and known papillotoxins clearly demonstrated a reduction in cell viability that varied with bath exposure time and type of agent tested. While only phenylbutazone and mefenamic acid produced significant cytotoxicity after a 24 h incubation period, cell viability assessed using the MTT

assay was only profoundly reduced by aspirin and paracetamol following sub-chronic exposure for 7 days. The rank order of cytotoxicity observed in hMIC was phenylbutazone > mefenamic acid > aspirin > paracetamol. The results demonstrate the potential of hMIC for investigating and defining the early cellular events in the pathogenesis of analgesic nephropathy.

Zager RA, Sacks BM, Burkhart KM, Williams AC. **Plasma membrane phospholipid integrity and orientation during hypoxic and toxic proximal tubular attack.** Kidney Int 1999;56(1):104-17.
BACKGROUND: Acute cell injury can activate intracellular phospholipase A2 (PLA2) and can inhibit plasma membrane aminophospholipid translocase(s). The latter maintains inner/outer plasma membrane phospholipid (PL) asymmetry. The mechanistic importance of PLA2-mediated PL breakdown and possible PL redistribution ("flip flop") to lethal tubule injury has not been well defined. This study was performed to help clarify these issues. METHODS: Proximal tubule segments (PTS) from normal CD-1 mice were subjected to either 30 minutes of hypoxia, Ca2+ ionophore (50 microM A23187), or oxidant attack (50 microM Fe). Lethal cell injury [the percentage of lactate dehydrogenase (LDH) release], plasma membrane PL expression [two-dimensional thin layer chromatography (TLC)], and free fatty acid (FFA) levels were then assessed. "Flip flop" was gauged by preferential decrements in phosphatidylserine (PS) versus phosphatidylcholine (PC; PS/PC ratios) in response to extracellular (Naja) PLA2 exposure. RESULTS: Hypoxia induced approximately 60% LDH release, but no PL losses were observed. FFA increments suggested, at most 3% or less PL hydrolysis. Naja PLA2 reduced PLs in hypoxic tubules, but paradoxically, mild cytoprotection resulted. In contrast to hypoxia, Ca2+ ionophore and Fe each induced significant PL losses (6 to 15%) despite minimal FFA accumulation or cell death (26 to 27% LDH release). Arachidonic acid markedly inhibited PLA2 activity, potentially explaining an inverse correlation (r = -0.91) between tubule FFA accumulation and PL decrements. No evidence for plasma membrane "flip flop" was observed. In vivo ischemia reperfusion and oxidant injury (myohemoglobinuria) induced 0 and 24% cortical PL depletion, respectively, validating these in vitro data. CONCLUSIONS: (a) Plasma membrane PLs are well preserved during acute hypoxic/ischemic injury, possibly because FFA accumulation (caused by mitochondrial inhibition) creates a negative feedback loop, inhibiting intracellular PLA2. (b) Exogenous PLA2 induces PL losses during hypoxia, but decreased cell injury can result. Together these findings suggest that PL loss may not be essential to hypoxic cell death. (c) Oxidant/Ca2+ overload injury induces early PL losses, perhaps facilitated by ongoing mitochondrial FFA metabolism, and (d) membrane "flip flop" does not appear to be an immediate mediator of acute necrotic tubular cell death.

IMMUNOTOXICITY

Beaman JR, Finch R, Gardner H, Hoffmann F, Rosencrance A, Zelikoff JT. **Mammalian immunoassays for predicting the toxicity of malathion in a laboratory fish model.** J Toxicol Environ Health 1999;56(8):523-42.
This study describes the use of a panel of immune assays, originally developed by the National Toxicology Program for assessing xenobiotic-induced immunotoxicity in mice, to quantify the effects of sublethal malathion exposure on the immune responses of fish. For this study, Japanese medaka (Oryzias latipes) were exposed subchronically to the organophosphate pesticide malathion in a series of

two experiments. In the first set of studies, fish were exposed for 7 or 14 d to untreated well water (i.e., controls) or to waterborne malathion at 0.2 or 0.8 mg/L. Following exposure, fish from each group were sacrificed and their kidneys (primary organ of leukopoiesis in fish and equivalent to mammalian bone marrow) were used to provide cells for assessing any malathion-induced effects upon nonspecific and acquired immune defense mechanisms. Effects upon humoral-mediated immunity were determined by enumerating antibody plaque-forming cell (PFC) numbers from a subset of fish exposed to malathion for 14 d and then injected intraperitoneally (ip) with sheep erythrocytes (sRBC). Results of these studies demonstrated that while malathion exposure had no significant effect upon hematocrit/leukocrit values or upon mitogen-stimulated T-cell lymphoproliferation, PFC numbers in the kidney of exposed fish were significantly reduced (compared to control fish) in a dose-dependent manner. In addition, total recoverable kidney cell numbers and viability, as well as superoxide anion production by kidney phagocytes, were reduced slightly (compared to control values) in fish exposed for 14 d to the highest malathion concentration tested. In the second set of experiments, medaka exposed for up to 21 d to either 0.1 or 0.3 mg malathion/L were challenged ip with an LD50 dose of the bacterial fish pathogen Yersinia ruckeri. Results from these infectivity studies demonstrated that exposure to either malathion concentration, for 14 or 21 d reduced host resistance against Yersinia infection. Taken together, these findings demonstrate the applicability of mammalian immune assays for predicting malathion-induced immunosuppression in a teleost fish, as well as the potential utility of a small laboratory fish to serve as an alternate model for mammals in immunotoxicological studies.

Blaikie L, Basketter DA. **Experience with a mouse intranasal test for the predictive identification of respiratory sensitization potential of proteins.** Food Chem Toxicol 1999;37(8):889-96.
The predictive identification of respiratory allergenic potential is an important primary step in the safety evaluation of (novel) proteins, such as the enzymes used in a range of consumer laundry products. In the past this has been achieved by assessing the relative ability of proteins to give rise to the formation of anaphylactic antibody in the guinea pig. Recently, an alternative model has been proposed which assesses the formation of specific IgG1 antibody in a mouse intranasal test (MINT), the assumption being that specific IgG1 antibody is a surrogate for anaphylactic antibody in the mouse. This procedure has undergone successful initial intralaboratory and interlaboratory assessment. In the present work, the MINT has been evaluated in a more thorough intralaboratory study using eight enzymes plus ovalbumin. While the data generated with a reference enzyme protein, Alcalase, showed good reproducibility, results with the remaining eight proteins led to estimates of their relative antigenic or sensitization potential several of which were at variance from those derived from the guinea pig/ human experience. In consequence, it is concluded that the MINT requires substantial further investigation before it can be adopted as a model for the assessment of the relative ability of proteins to behave as respiratory allergens.

Blaikie L, Basketter DA. **Strain variation in the IgG1 antibody response to proteins administered intranasally in the mouse.** Food Chem Toxicol 1999;37(8):897-904.
Proteins, including enzymes, have the potential to behave as respiratory allergens. In consequence, guinea pig methods have been developed which permit an assessment to be made of their respiratory allergenic/antigenic potential relative to an appropriate reference substance. Recently, a murine model, the mouse intranasal test (MINT) has been proposed as a potential alternative. However, to be of value,

the new method should give a rank order of relative potency for a range of proteins which correlates with that found in guinea pig models and in human experience. Using the mouse strain recommended for the MINT, BDF1, in an extensive intralaboratory assessment, the relative potency of several of the eight proteins used was at variance with that expected from the historic data. Where genetic factors are important, as in the assessment of antigenicity, the rank order for a range of proteins in a particular inbred or F1 hybrid strain may not reflect that in humans. To examine whether the earlier observations were a strain rather than a species dependent phenomenon, five proteins of varying antigenic potency previously tested using the BDF1 strain were selected and tested using the MINT protocol in BALB/c, CBA/Ca and CB6F1 inbred/F1 hybrid strains, as well as in the outbred Swiss S strain. The results clearly indicated that the relative potency of the proteins was dependent on the mouse strain used and thus with haplotype. When assessed against the standard reference enzyme, Alcalase (a process used for the establishment of occupational exposure guidelines), the rank order was strain dependent and results from none of the mouse strains would have led to similar conclusions to those derived from existing models and the human epidemiological data. Based on the presently available information, it is not possible to be certain that any mouse model reliant on the responsiveness of a particular strain (including the MINT) might not lead to an incorrect estimation of respiratory antigenic and thus allergenic potency. In consequence, the MINT may not be viable as a model for the assessment of the relative ability of proteins to behave as respiratory allergens.

Cederbrant K, Gunnarsson LG, Hultman P, Norda R, Tibbling-Grahn L. **In vitro lymphoproliferative assays with HgCl2 cannot identify patients with systemic symptoms attributed to dental amalgam.** J Dent Res 1999;78(8):1450-8.
Dental amalgam is suspected, by some exposed individuals, to cause various systemic psychological, sensory, and neurological symptoms. Since not all amalgam-bearers experience such reactions, an individual characteristic--for example, a susceptible immune system--might explain these conditions. In vitro lymphocyte proliferation is a valuable tool in the diagnosis of allergy. With HgCl2 as the antigen, however, the test is hampered, because Hg2+ can cause unspecific lymphocyte proliferation, optimal at 1.4 to 9.5 micrograms HgCl2/mL. Recently, the use of suboptimal HgCl2 concentrations (< or = 0.5 microgram/mL) has been suggested to circumvent these problems. The main aim of this study was to investigate whether patients with systemic symptoms alleged to result from the presence of dental amalgam differ from healthy controls, with reference to in vitro lymphoproliferative responses to HgCl2 < or = 0.5 microgram/mL. Three different test protocols--lymphocyte transformation test (LTT) in micro- and macro-cultures, and the memory lymphocyte immunostimulation assay (MELISA)--were used. Other immune parameters--such as a standard patch test for dental materials, the number of T- and B-lymphocytes, monocytes, granulocytes, and NK cells in peripheral blood, allergic symptoms, and predisposition--were also investigated. Twenty-three amalgam patients, 30 healthy blood donors with amalgam, ten healthy subjects without amalgam, and nine patients with oral lichen planus (OLP) adjacent to dental amalgam and a positive patch test to Hg0 were tested. None of the investigated immune parameters revealed any significant differences between amalgam patients and controls. The sensitivity of in vitro lymphocyte proliferation ranged from 33 to 67%, with the OLP patients as a positive control group, and the specificity from 0 to 70% for healthy controls with a negative patch test to Hg0. Thus, despite the use of HgCl2 < or = 0.5 microgram/mL, a high frequency of positive results was obtained among healthy subjects with or without dental amalgam. Consequently, in vitro

lymphocyte proliferation with HgCl2 cannot be used as an objective marker for mercury allergy in dental amalgam-bearers.

Coutant KD, Ulrich P, Thomas H, Cordier A, Brugerolle De Fraissinette A. **Early changes in murine epidermal cell phenotype by contact sensitizers.** Toxicol Sci 1999;48(1):74-81.
In order to develop an in vitro predictive assay for the detection of contact sensitizers, we investigated the possible modulation of the expression of cell-surface molecules in the early phases of treatment of murine epidermal cells (EC) with known contact sensitizers. After in vitro treatment of Balb/c EC with the strong contact sensitizer, TNBS, Langerhans cells (LCs) demonstrated a rapid up-regulation of CD45, CD40, CD32/16 (Fc gamma RII/III) and CD23 (Fc epsilon RII) molecules. CD45 and CD40 were also rapidly up-regulated on the dendritic epidermal T cells. Interestingly, after treatment with this severe sensitizer, a marked induction of CD40 expression was found on a CD45 negative population, most probably keratinocytes. In contrast to these cell-surface molecules, I-Ad/I-Ed and CD90.2 expression were unchanged. No change was observed on the expression of CD45 and CD40 after treatment with a mild or a weak contact sensitizer, citral and citronellal respectively. In contrast, like TNBS, they up-regulated the expression of CD32/16 and CD23 on LCs. The irritant sodium dodecyl sulfate had no effect on all these cell-surface molecules. Our results indicated that in vitro, chemicals with allergic potential induced early specific phenotype changes that may represent an early-activated state of the cells. This state may be responsible for initiating the afferent phase of contact sensitivity in vivo. Based on these findings, it might be possible to develop an in vitro assay to reduce the number of experimental animals for a fast screening of contact sensitizers and for discriminating between mild contact sensitizers and irritants.

Daffern PJ, Jagels MA, Saad JJ, Fischer W, Hugli TE. **Upper airway epithelial cells support eosinophil survival in vitro through production of GM-CSF and prostaglandin E2: regulation by glucocorticoids and TNF-alpha.** Allergy Asthma Proc 1999;20(4):243-53.
Production of GM-CSF by epithelial cells has been implicated in eosinophil survival within the airways, although GM-CSF promotes neutrophil and monocyte survival as well. Using primary cultures of human airway epithelial cells, we undertook a comprehensive examination of factors that enhance eosinophil survival or apoptosis. Unstimulated epithelial cells were compared to epithelial cells stimulated with TNF-alpha in the presence or absence of dexamethasone. A striking increase in survival was observed when peripheral blood eosinophils were cultured with supernatants derived from unstimulated and TNF-alpha-stimulated epithelial cells. Cultured epithelial cells were examined for transcripts of cytokines shown to enhance eosinophil survival (GM-CSF, IL-3, IL-5, IL-13, and IFN-gamma), and transcripts for cytokines promoting apoptosis (IL-10 and TGF-beta). GM-CSF transcripts, but not the other cytokines, were present in unstimulated epithelial cells, and levels were increased with TNF-alpha stimulation. TNF-alpha stimulation increased the levels of GM-CSF and PGE2 in epithelial cell supernatants and dexamethasone suppressed the TNF-alpha induced increases. The survival effects of the TNF-alpha-stimulated supernatants were effectively blocked by neutralizing antibodies to GM-CSF or by dexamethasone treatment of epithelial cells. Selectivity of GM-CSF for eosinophil versus neutrophil survival was demonstrated and suggests that epithelial cell regulation of GM-CSF and PGE2 contribute to eosinophil survival in vitro and may contribute to eosinophil accumulation in allergic disease.

Di Stasi LC, Gomes JC, Vilegas W. **Studies on anti-allergic constituents in the leaves and stems of**

Anchientia salutaris var. martiana (Violaceae). Chem Pharm Bull (Tokyo) 1999;47(6):890-3.
The anti-allergic active fractionation of hexane extracts of the leaves and stems of Anchietia salutaris var. martiana (family Violaceae) was performed by monitoring their activities with an in vitro bioassay system measuring the inhibitory effects on induced histamine release from guinea pig lung cells. Three known pentacyclic triterpenes (friedelin, alpha-amyrin, beta-amyrin) were isolated, but these compounds were inactive. Aliphatic hydrocarbons and methyl esters of fatty acids (palmitic, oleic, linoleic, linolenic acids) were detected in active fractions. All compounds isolated were detected for the first time in this medicinal plant.

Diel F, Horr B, Borck H, Savtchenko H, Mitsche T, Diel E. **Pyrethroids and piperonyl-butoxide affect human T-lymphocytes in vitro.** Toxicol Lett 1999;107(1-3):65-74.
Synthetic pyrethroids are increasingly used as insecticides and are claimed to have a relatively low human toxicity. The aim of this study was to examine the in vitro effects of the synthetic pyrethroid S-bioallethrin alone and in combination with the common synergist piperonyl-butoxide (PBO) on human blood lymphocytes and basophils in atopic individuals and non-atopic control subjects. S-bioallethrin and PBO also caused inhibition of lymphocyte proliferation (MTT-test) after a 72-h culture period in a concentration dependent manner. In contrast to the MTT-measurements the combined agents are more effective in inhibiting interleukin-4 (IL-4)- and interferon-gamma (IFN-gamma)-production. The regulatory IL-4/IFN-gamma balance showed a significant difference between atopic and non-atopic subjects after a culture period of 24-48 h in the presence of micromolar S-bioallethrin ($P < 0.001$). Furthermore S-bioallethrin, PBO and the combined agents induced histamine release from human basophils. Although this effect was little compared to histamine liberators like FMLP and anti-IgE, the response to S-bioallethrin and PBO was significantly different in atopic donors compared with non-atopics ($P < 0.01$). In scratch test experiments 4 of 18 tested atopic volunteers showed positive reaction (wheals and flares) to S-bioallethrin and permethrin, whereas no reaction could be measured in the control group (age-matched). These findings demonstrate the immuno- and allergo-toxicological properties of the synthetic pyrethroid S-bioallethrin combined with the synergistic PBO using this in vitro approach with human lymphocytes and basophils.

Gasiorowski K, Brokos JB, Szyba K, Wozniak D, Fraser DM, Zakeeruddin SM, Graetzel M. **Evaluation of genotoxic and immunotoxic activities of potential glucose biosensor components: ferrocenes.** Biometals 1999;12:19-26.
Three ferrocenes used in glucose biosensor construction were tested in the aspect of genotoxic and immunotoxic activities. All three ferrocenes were not mutagenic in the standard bacterial Ames test. Equally in the Sister Chromatid Exchanges test in human lymphocyte cultures, the genotoxic action of tested ferrocenes could be excluded. However, all three significantly decreased the rate of lymphocyte proliferation and especially diminished the numbers of B-lymphocytes and NK-cells after 72 hours of in vitro culture. Marked differences between the ferrocenes in their immunotoxic activities were noticed, and we were able to select those which would be relatively safe and those which should be avoided in further investigation of the glucose biosensor construction. Our results indicate the necessity to estimate immunotoxic effects as well as genotoxic effects, especially in biosensor components potentially used in vivo.

Hansen LA, Poulsen OM, Wurtz H. **Endotoxin potency in the A549 lung epithelial cell bioassay and**

the limulus amebocyte lysate assay. J Immunol Methods 1999;226(1-2):49-58.
The purpose of this study was to elucidate to what extent the potency of endotoxins measured by the limulus amebocyte lysate (LAL) assay is reflected in the potency in an in vitro assay based on release of interleukin-8 (IL-8) from a lung epithelial cell line, A549. Lipopolysaccharides (LPS) from Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis and detoxified LPS from E. coli were applied in serial dilutions in the LAL assay and in the A549 bioassay. Also 19 organic dust samples from waste recycling plants were tested. The A549 cells were incubated for 24 h with LPS or dust, and the IL-8 secretion was determined by ELISA. The method for evaluation of the LAL assay showed linearity for the four endotoxins. Using the slope as a measure of the potency factor (PF), LPS from E. coli and S. enteritidis was about four times more potent than that for LPS from K. pneumoniae and P. aeruginosa. In the A549 bioassay each of the different types of endotoxin had characteristic and very different dose-response curves. The potency of the LPS, in the A549 bioassay, ranked as follows K. pneumoniae > P. aeruginosa > E. coli > or = S. enteritidis. The content of endotoxin in the dust samples did not correlate with their potency in the A549 bioassay. The present study indicates a poor correlation between the potency of endotoxin in the LAL assay compared with the A549 bioassay. The lack of correlation when organic dust samples are tested may reflect the fact that these samples contain biological active compounds, which are non-reactive in the LAL-assay but stimulate IL-8 secretion from epithelial cells.

Hoffmann A, Jamin A, Foetisch K, May S, Aulepp H, Haustein D, Vieths S. **Determination of the allergenic activity of birch pollen and apple prick test solutions by measurement of beta-hexosaminidase release from RBL-2H3 cells. Comparison with classical methods in allergen standardization.** Allergy 1999;54(5):446-54.
BIOSIS COPYRIGHT: BIOL ABS. Background: A murine in vitro model of the allergic type I reaction was set up to determine the biologic activity of extracts without involvement of human beings. It is based on beta-hexosaminidase release from passively sensitized RBL cells after allergen challenge. The intended application of this RBL cell assay in the field of quality control of allergenic extracts requires its comparison with established methods. Methods: The activity of five standardized birch-pollen prick test solutions wa s observed, presumably because a reduced number of epitopes were recognized by the monospecific reagin. In contrast to standardized birch-pollen extracts, nonstandardized apple extracts showed poor activity in all assays. Conclusions: This murine model might be a useful tool in the quality control of allergenic extracts. It combines properties of assays based on standardized antisera and of assays that consider IgE cross-linking properties.

Kimber I, Pichowski JS, Basketter DA, Dearman RJ. **Immune responses to contact allergens: novel approaches to hazard evaluation.** Toxicol Lett 1999;106(2-3):237-46.
Progress in our understanding of the immunobiological mechanisms that cause skin sensitization and allergic contact dermatitis has facilitated consideration of alternative approaches to hazard evaluation. One such is the murine local lymph node assay in which, in contrast to more traditional guinea pig tests, sensitizing activity is measured as a function of events associated with the induction, rather than the elicitation, phase of contact hypersensitivity. Activity in the local lymph node assay is dependent upon all of those immunological events that are initiated following first encounter with chemical allergen and which result in the stimulation of T lymphocyte proliferative responses in lymph nodes draining the site

of exposure. In this respect the assay embraces in an holistic way the induction of skin sensitization. With the objective of developing in vitro approaches to hazard identification, consideration has been given to discrete immunological responses that characterize the induction of skin sensitization. Most attention has focused upon the changes induced by chemical allergens in the phenotype and function of epidermal Langerhans cells and in cytokine expression. In addition, attempts have been made to identify contact allergens as a function of their ability to provoke in vitro specific responses by unprimed T lymphocytes. These novel approaches to skin sensitization testing and their potential utility in the context of toxicological evaluations are reviewed in this article.

Manetz TS, Meade BJ. **Development of a flow cytometry assay for the identification and differentiation of chemicals with the potential to elicit irritation, IgE-mediated, or T cell-mediated hypersensitivity responses.** Toxicol Sci 1999;48(2):206-17.
These studies were conducted to investigate the potential use of a flow cytometric analysis method for the identification and differentiation of chemicals with the capacity to induce irritation, IgE- or T cell-mediated hypersensitivity responses. An initial study investigated the ability of equally sensitizing concentrations (determined by local lymph node assay) of IgE-mediated (Toluene Diisocyanate-TDI) and T cell-mediated (Dinitrofluorobenzene-DNFB) allergens to differentially modulate the IgE+B220+ population in the lymph nodes draining the dermal exposure site. Sodium lauryl sulfate (SLS) was also tested as a nonsensitizing irritant control. Female B6C3F1 mice were dermally exposed once daily for 4 consecutive days, with the optimum time point for analysis determined by examining the IgE+B220+ population 8, 10, and 12 days post-initial chemical exposure. At the peak time point, day 10, the IgE +B220+ population was significantly elevated in TDI (41%), while moderately elevated in DNFB (18%) exposed animals when compared to the vehicle (0.8%), and remained unchanged in SLS (2.2%) exposed animals when compared to the ethanol control (2.5%). Experiments in our laboratory and others have demonstrated that the draining lymph node B220+ population becomes significantly elevated following exposure to allergens (IgE- and T cell-mediated), not irritants, allowing for their differentiation. An existing mouse ear swelling assay was used to identify chemical irritants. Therefore, using the endpoints of percent ear swelling, percent B220+ cells, and percent IgE+B220+ cells, a combined irritancy/ phenotypic analysis assay was developed and tested with tetradecane (irritant), toluene diisocyanate, trimellitic anhydride (IgE-mediated allergens), benzalkonium chloride, dinitrofluorobenzene, oxazolone, and dinitrochlorobenzene (T cell-mediated allergens) over a range of concentrations. Based upon the pattern of response observed, a paradigm was developed for continued evaluation: Irritant exposure will result in significant ear swelling without altering the B220+ or IgE+B220+ populations. Exposure to sensitizers (IgE-mediated or T cell-mediated) will increase the B220+ population and the percent ear swelling will remain unchanged or will significantly increase, depending on the irritancy capacity of the chemical. Both the IgE+B220+ and B220+ populations will become elevated at the same test concentration following exposure to IgE-mediated, hypersensitivity inducing allergens. At its peak, the percent of IgE+B220+ cells will be equal to the percent of B220+ cells. The B220+ population will increase at a lower test concentration than the IgE+B220+ population, following exposure to T cell-mediated, hypersensitivity inducing allergens. At its peak, the percent of IgE+B220+ cells will reach less than half that of the percent of B220+ cells. The irritancy/phenotypic analysis method may represent a single murine assay able to identify and differentiate chemicals with the capacity to induce irritation, or IgE-mediated or T cell-mediated responses.

Mistry N, Evans MD, Griffiths HR, Kasai H, Herbert KE, Lunec J. **Immunochemical detection of glyoxal DNA damage.** Free Radic Biol Med 1999;26(9-10):1267-73.
The relevance of reactive oxygen species (ROS) in the pathogenesis of inflammatory diseases is widely documented. Immunochemical detection of ROS DNA adducts has been developed, however, recognition of glyoxal-DNA adducts has not previously been described. We have generated a polyclonal antibody that has shown increased antibody binding to ROS-modified DNA in comparison to native DNA. In addition, dose-dependent antibody binding to DNA modified with ascorbate alone was shown, with significant inhibition by desferrioxamine, catalase, and ethanol. Minimal inhibition was observed with uric acid, 1,10-phenanthroline and DMSO. However, antibody binding in the presence of EDTA increased 3500-fold. The involvement of hydrogen peroxide and hydroxyl radical in ascorbate-mediated DNA damage is consistent with ascorbate acting as a reducing agent for DNA-bound metal ions. Glyoxal is known to be formed during oxidation of ascorbate. Glyoxylated DNA, that previously had been proposed as a marker of oxidative damage, was recognised in a dose dependent manner using the antibody. We describe the potential use of our anti-ROS DNA antibody, that detects predominantly Fenton-type mediated damage to DNA and report on its specificity for the recognition of glyoxal-DNA adducts.

Mordvinov VA, Schwenger GT, Fournier R, De Boer ML, Peroni SE, Singh AD, Karlen S, Holland JW, Sanderson CJ. **Binding of YY1 and Oct1 to a novel element that downregulates expression of IL-5 in human T cells.** J Allergy Clin Immunol 1999;103(6):1125-35.
BACKGROUND: IL-5 controls development of eosinophilia and has been shown to be involved in the pathogenesis of allergic diseases. In both atopic and nonatopic asthma, elevated IL-5 has been detected in peripheral blood and the airways. IL-5 is produced mainly by activated T cells, and its expression is regulated at the transcriptional level. OBJECTIVE: This study focuses on the functional analysis of the human IL-5 (hIL-5) promoter and characterization of cis -regulatory elements and transcription factors involved in the suppression of IL-5 transcription in T cells. METHODS: Methods used in this study include DNase I footprint assays, electrophoretic mobility shift assays, and functional analysis by mammalian cell transfection involving deletion analysis and site-directed mutagenesis. RESULTS: We identified 5 protein binding regions (BRs) located within the proximal hIL-5 promoter. Functional analysis indicates that the BRs are involved in control of hIL-5 promoter activity. Two of these regions, BR3 and BR4 located at positions -102 to -73, have not previously been described as regulators of IL-5 expression in T cells. We show that the BR3 sequence contains a novel negative regulatory element located at positions -90 to -79 of the hIL-5 promoter, which binds Oct1, octamer-like, and YY1 nuclear factors. Substitution mutations, which abolished binding of these proteins to the BR3 sequence, significantly increased hIL-5 promoter activity in activated T cells. CONCLUSION: We suggest that Oct1, YY1, and octamer-like factors binding to the -90/-79 sequence within the proximal IL-5 promoter are involved in suppression of IL-5 transcription in T cells.

Rizova H, Carayon P, Barbier A, Lacheretz F, Dubertret L, Michel L. **Contact allergens, but not irritants, alter receptor-mediated endocytosis by human epidermal Langerhans cells.** Br J Dermatol 1999;140(2):200-9.
BIOSIS COPYRIGHT: BIOL ABS. Allergic contact dermatitis is a T-cell-mediated inflammation, induced by contact with sensitizers and occurring through the release of epidermal cytokines and the

activation of epidermal Langerhans cells (LCs). The aim of this study was to analyse early events of LC activation induced either by contact allergens or by irritants devoid of any contact allergenic properties, in order to obtain an in vitro method to discriminate between these two groups of molecules. Various contact sensitizers and o 37~C. Pre-incubation of LCs with either sensitizers or irritants increased the spontaneous internalization of HLA-DR molecules with a similar magnitude, but no clear discrimination between sensitizer and irritant effects was obtained by flow cytometry analysis. In contrast, confocal microscopy enabled discrimination between the effects of sensitizers and irritants: sensitizer-treated samples showed internalized HLA-DR molecules aggregated in large vesicles with very bright fluorescence; irrita these molecules in the prelysosomes only near the cell membrane. We conclude that contact allergens and irritants induce distinct patterns of HLA-DR endocytosis, which may be useful for the development of in vitro screening tests.

Siwicki AK, Morand M, Studnicka M. **In vitro immunotoxicology: practical application.** Acta Vet Brno 1998;67(4):303-7.
BIOSIS COPYRIGHT: BIOL ABS. The in vitro immunology is one of the most important directions in immunotoxicology. At present, many in vitro methods are used in control drug discovery, agents for chemotherapeutics and for monitoring of environmental contamination. In this paper, we present various methods which could by used for examination of the influence of different environmental chemicals and other xenobiotics on the immunocompetent cell activity in fish. The chemiluminescence response assay is important for study the phagocyte activity and the proliferative response assay of lymphocytes stimulated by mitogens is very important method for study the effects of xenobiotics on the lymphocyte T and B activities. We also demonstrate that in vitro immune response assay analyzed by PFC and ELISPOT are very sensitive techniques for studies of the effects of xenobiotics on the antibody secreting cells.

Slauson SD, Silva HT, Sherwood SW, Morris RE. **Flow cytometric analysis of the molecular mechanisms of immunosuppressive action of the active metabolite of leflunomide and its malononitrilamide analogues in a novel whole blood assay.** Immunol Lett 1999;67(3):179-83.
Malononitrilamides (MNAs) are a new class of immunomodulatory drug highly effective in in vivo models of allo- and xenotransplantation. Knowledge of their effects on immune cells, however, is limited and has been derived solely from investigations using isolated mononuclear cells. This use of purified cells to investigate drug activity is not ideal, so we have combined the analytical power of flow cytometry with our mitogen-driven, whole blood lymphocyte activation and proliferation assays to investigate the in vitro mechanism of action of MNAs. We first show that MNAs (A77 1726, HMR1279, and HMR1715), as well as brequinar (BQR) and cyclosporine (CsA), effectively inhibit cell activation antigen expression and lymphocyte proliferation. We next show that the inhibitory effects of MNAs and BQR, but not CsA, are reversed by the addition of uridine to the culture. These results suggest that inhibition of pyrimidine biosynthesis may be a mechanism by which MNAs suppress both lymphocyte activation and proliferation since these effects were reversed when uridine nucleotide pools were replenished. This novel finding of suppression of activation antigen expression by MNAs in whole blood expands our understanding of the effects of this new class of drug.

Thuvander A, Wikman C, Gadhasson I. **In vitro exposure of human lymphocytes to trichothecenes: individual variation in sensitivity and effects of combined exposure on lymphocyte function.** Food

Chem Toxicol 1999;37(6):639-48.
The trichothecenes are mycotoxins produced by fungi of the genus Fusarium, which are commonly present in foods and feed of cereal origin. Owing to the lack of sufficient toxicological data for most of the trichothecenes, in vitro studies may contribute to risk assessments of these toxins. In the present report, human lymphocyte cultures were used to study the individual variation in sensitivity among humans and the effects on in vitro Ig production. Furthermore, proliferative responses of cells exposed to combinations of two of the toxins were studied. Four toxins, T-2 toxin, diacetoxyscirpenol (DAS), nivalenol (NIV) and deoxynivalenol (DON) were included in the study. All four of the tested trichothecenes effectively inhibited mitogen-induced lymphocyte proliferation. There were no statistically significant differences in sensitivity to the toxins between lymphocytes from female and male blood donors. The individual variation in sensitivity, evaluated as the range of IC50 values, was rather limited (within a factor of 3 to 4). Immunoglobulin production by pokeweed-stimulated human lymphocytes was also effectively inhibited with IC50 values similar to the IC50 values in the proliferation tests for DON and NIV. However, IC50 values for Ig synthesis in cultures exposed to T2 were approximately two to three times higher than the corresponding IC50 values found in the proliferation tests. At low levels of exposure, elevated Ig production was observed in lymphocyte cultures from four out of the five blood donors tested. This effect was most pronounced on IgA synthesis. Combinations of NIV with T2, DAS or DON resulted in additive toxicity in the lymphocyte proliferation test, while combinations of DON with T2 or DAS resulted in an inhibition that was slightly lower than what could have been expected from the inhibition produced by the individual toxins. In conclusion, the tested trichothecenes inhibited both proliferation and Ig production in human lymphocytes in a dose-dependent manner with limited variation in sensitivity between individuals. Enhanced Ig production was observed in cell cultures exposed to the lower doses of the toxins. Combined exposure to two of the toxins resulted mainly in additive or antagonistic effects, although synergistic effects cannot be excluded and should be further investigated. These findings indicate that the total intake of type A and B trichothecenes should be taken into account in risk assessments.

Vamnes JS, Gjerdet NR, Morken T, Moe G, Matre R. **In vitro lymphocyte reactivity to gold compounds in the diagnosis of contact hypersensitivity.** Contact Dermatitis 1999;41(3):156-60.
The use of the lymphocyte transformation test (LTT) in the diagnosis of contact hypersensitivity to gold was studied in 8 patients who had positive patch tests to gold salts, and in 8 control subjects who were negative to such patch tests. Gold sodium thiosulfate and gold chloride were added to cultures of lymphocytes, which were labeled by 3H-thymidine after 96 h. The lymphocyte stimulation index was calculated as the beta-counts in stimulated cultures divided by those in control cultures. The index was statistically significantly higher for the patient group (p=0.005-0.04) than for the control group. Levels of interferon-gamma (IFN-gamma) were determined for the supernatants of the lymphocyte cultures. An index IFN-gamma, which is defined as the level of IFN-gamma in stimulated cultures divided by that in control cultures, was statistically significantly higher for the patient group (p=0.01-0.006). The LTT stimulation index showed specificity and sensitivity between 67 and 80%, the respective values for Index IFN-gamma being between 73 and 100% when the patch test was used as a reference method. Evaluation of lymphocyte reactivity might be of future interest in the diagnosis of allergic reactions to gold if the sensitivity and specificity can be improved.

Whalen MM, Loganathan BG, Kannan K. **Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells in vitro.** Environ Res 1999;81(2):108-16.
The widespread environmental contamination, bio-accumulation, and toxic effects of butyltins (BTs) in wildlife is well documented, but the role of BTs in debilitating human immune function mediated through natural killer (NK) lymphocytes (a primary immune defense against tumor and virally infected cells) has not been described. In this study, we assessed the effects of in vitro exposure to a range of concentrations (encompassing environmentally relevant concentrations) of MBT, DBT, and TBT on human natural killer lymphocytes obtained from adult male and female donors. TBT inhibited the tumor-killing capacity of NK cells when the NK cells were pretreated in vitro at 200 nM for as little as 1 h. Inhibition of NK cytotoxic function ranged from 40 to greater than 90%. The toxic potential of butyltins followed the order of TBT > DBT > MBT. Conjugation assays revealed that after a 24-h exposure to TBT, there was about a 50% decrease in NK cell binding to tumor cells, indicating alteration of the NK cell receptors for tumor cells. Analysis of whole-blood samples for BTs revealed the presence of detectable concentrations of MBT, DBT, and TBT in all of the donors, indicating possible exposure of NK cells to BTs in the blood. The results of this study provide evidence that butyltin compounds significantly inhibit NK cell function and possible NK cell-mediated immunotoxic potential in humans. Copyright 1999 Academic Press.

Yucesoy B, Mirshahidi S, Yucesoy C, Karakaya A. **In vitro effects of various metals on natural killer cell activity in cultured human lymphocytes.** Immunopharmacol Immunotoxicol 1999;21(3):599-607.
Heavy metals have been shown to have a differential effects on various aspects of immune response. Recently natural killer cells have been widely investigated due to their purported role in immune surveillance. To ascertain the immunotoxic effects of lead, cadmium, nickel and chromium on natural killer (NK) cell activity in vitro, peripheral blood lymphocytes from normal donors were examined in the presence of different concentrations (10(-5)-10(-8) M) of four selected metal salts (cadmium sulphate, lead nitrate, chromium nitrate and nickel sulphate). NK cell activity was evaluated in a 4-h chromium release assay against K562 target cells. All of the metal salts were found to exert no effect on NK cell function in the human concentration range.

NEUROTOXICITY

Bruinink A, Rasonyi T, Sidler C. **Differences in neurotoxic effects of ochratoxin A, ochracin and ochratoxin-alpha in vitro.** Nat Toxins 1998;6(5):173-7.
The mycotoxin ochratoxin A (OTA) is a chlorinated dihydroisocoumarin derivative connected through an amide-bond to L-phenylalanine. In a previous study we could show that competition with L-phenylalanine-dependent processes does not play a role in OTA neurotoxicity. To test whether the isocoumarin part is responsible for the neurotoxic effects, we determined in the present study the effects of the hydrolysis product of OTA, ochratoxin-alpha (OTalpha), and of ochracin on embryonic chick brain cell cultures. In addition, we investigated the interaction between OTA and ochracin regarding the neurotoxic effects. We report here that OTalpha did not affect brain cell cultures at concentrations up to 15 microM. With the exception of a small (20%) but significant reduction in cell culture, cellular protein at concentrations above 0.3 microM, in our cell cultures' cell function, as defined by neutral red uptake

and MTT-dehydrogenase activity, was only reduced by high OTalpha concentrations (1 mM). Addition of 0.1 microM OTA increased ochracin cytotoxicity as defined by latter parameters. No effects on cell culture NF68kD content could be detected. The results are discussed with regard to the existence of an OTA target interaction binding site. Copyright 1998 John Wiley & Sons, Ltd.

Bruno V, Battaglia G, Kingston A, O'Neill MJ, Catania MV, Di Grezia R, Nicoletti F. **Neuroprotective activity of the potent and selective mGlu1a metabotropic glutamate receptor antagonist, (+)-2-methyl-4 carboxyphenylglycine (LY367385): comparison with LY357366, a broader spectrum antagonist with equal affinity for mGlu1a and mGlu5 receptors.** Neuropharmacology 1999;38 (2):199-207.
(+)-2-Methyl-4-carboxyphenylglycine (LY367385), a potent and selective antagonist of mGlu1a metabotropic glutamate receptors, was neuroprotective in the following in vitro and in vivo models of excitotoxic death: (i) mixed cultures of murine cortical cells transiently exposed to N-methyl-D-aspartate (NMDA); (ii) rats monolaterally infused with NMDA into the caudate nucleus; and (iii) gerbils subjected to transient global ischemia. We have compared the activity of LY367385 with that of the novel compound (+/-)-alpha-thioxantylmethyl-4-carboxyphenylglycine (LY367366), which antagonizes both mGlu1a and -5 receptors at low micromolar concentrations, but also recruits other subtypes at higher concentrations. Although LY367366 was neuroprotective, it was in general less efficacious than LY357385, suggesting that inhibition of mGlu1 receptors is sufficient to confer significant neuroprotection. We conclude that endogenous activation of mGlu1a receptors (or perhaps other mGlu1 receptor splice variants) contributes to the development of neuronal degeneration of excitotoxic origin.

Calderon FH, Bonnefont A, Munoz FJ, Fernandez V, Videla LA, Inestrosa NC. **PC12 and neuro 2a cells have different susceptibilities to acetylcholinesterase-amyloid complexes, amyloid25-35 fragment, glutamate, and hydrogen peroxide.** J Neurosci Res 1999;56(6):620-31.
This work addresses the differential effects of several oxidative insults on two neuronal cell lines, PC12 and Neuro 2a cells, extensively used as neuronal models in vitro. We measured cellular damage using the cytotoxic assays for MTT reduction and LDH release and found that acetylcholinesterase (AChE)-amyloid-beta-peptide (Abeta) complexes, Abeta25-35 fragment, glutamate and H2O2 were over 200-fold more toxic to PC12 than to Neuro 2a cells. 17alpha and 17beta estradiol were able to protect both cell types from damage caused by H2O2 or glutamate. By contrast, other insults not related to oxidative stress, such as those caused by the nonionic detergent Triton X-100 and serum deprivation, induced a similar level of damage in both PC12 and Neuro 2a cells. Considering that the Abeta peptide, H2O2 and glutamate are cellular insults that cause an increase in reactive oxygen species (ROS), the intracellular levels of the antioxidant compound, glutathione were verified. Neuro 2a cells were found to have 4- to 5-fold more glutathione than PC12 cells. Our results suggest that Neuro 2a cells are less susceptible to exposure to AChE-Abeta complexes, Abeta25-35 fragment, glutamate and H2O2 than PC12 cells, due to higher intracellular levels of antioxidant defense factors.

Castagne V, Lefevre K, Natero R, Clarke PG, Bedker DA. **An optimal redox status for the survival of axotomized ganglion cells in the developing retina.** Neuroscience 1999;93(1):313-20.
The neuronal redox status influences the expression of genes involved in neuronal survival. We previously showed that antioxidants may reduce the number of dying ganglion cells following axotomy in chick embryos. In the present study, we show that various antioxidants, including the new spin trap

azulenyl nitrone and 1,3-dimethyl-2-thiourea, protect axotomized ganglion cells, confirming that neuronal death involves an imbalance of the cellular redox status towards oxidation. However, high concentrations of antioxidants did not protect ganglion cells, suggesting that excessive reduction is detrimental for neurons. Simultaneous injections of two different antioxidants gave results only partly supporting this view. Combinations of azulenyl nitrone and N-acetyl cysteine in fact gave greater protection than either antioxidant alone, whereas N-acetyl cysteine lost its neuroprotective effects and diminished those of alpha-phenyl-N-tert-butyl nitrone when the two compounds were injected simultaneously. The results of the combined treatments suggest that azulenyl nitrone and alpha-phenyl-N-tert-butyl nitrone do not have the same chemical effects within the ganglion cells. Moreover, N-acetyl cysteine's own antioxidant properties enhance the spin trapping effects of azulenyl nitrone but potentiate the toxicity of alpha-phenyl-N-tert-butyl nitrone. Our main conclusion is that neuronal survival requires the maintenance of the redox status near an optimal set-point. "Reductive stress" may be as dangerous as oxidative stress.

Griebel G, Perrault G, Tan S, Schoemaker H, Sanger DJ. **Pharmacological studies on synthetic flavonoids: comparison with diazepam.** Neuropharmacology 1999;38(7):965-77.
The present experiments compared the central BZ-omega binding characteristics and pharmacological profiles of two synthetic flavonoids (6-bromoflavone and 6-bromo-3'-nitroflavone) with those of the benzodiazepine (BZ) diazepam. In vitro experiments showed that while diazepam displaced [3H] flumazenil binding to the GABA(A) receptor in membranes from rat cerebellum and spinal cord, two brain areas enriched in the BZ-omega1 and BZ-omega2 receptor subtypes, with nearly equivalent half maximally effective concentrations, 6-bromo-3'-nitroflavone was somewhat more potent in displacing [3H]flumazenil binding to membranes from rat cerebellum (IC50 = 31 nM) than from spinal cord (IC50 = 120 nM), indicating selectivity for the BZ-omega1 receptor subtype. 6-Bromoflavone displayed weak (IC50 = 970 nM) affinity for the BZ-omega1 and no affinity for the BZ-omega2 (IC50 > 1000 nM) receptor subtypes. Diazepam, but not the synthetic flavonoids increased the latency to clonic seizures produced by isoniazid, thereby indicating that neither 6-bromoflavone nor 6-bromo-3'-nitroflavone display detectable intrinsic activity at GABA(A) receptors in vivo. Results from two conflict tests in rats showed that 6-bromoflavone (3-10 mg/kg) and 6-bromo-3'-nitroflavone (0.3-1 mg/kg) elicited anxiolytic-like activity in the punished drinking test, while both drugs were inactive in the punished lever pressing test. The positive effects displayed by the synthetic flavonoids in the punished drinking procedure were smaller than that of diazepam and were not antagonized by the BZ receptor antagonist flumazenil. In two models of exploratory activity, 6-bromoflavone (3-30 mg/kg) and 6-bromo-3'-nitroflavone (0.3-1 mg/kg) produced anxiolytic-like effects in the rat elevated plus-maze test, whereas both compounds failed to modify the behavior of mice in the light/dark test over a wide dose-range. The effects in the elevated plus-maze were antagonized by flumazenil. In the mouse defense test battery, where mice were confronted with a natural threat (a rat), 6-bromoflavone and 6-bromo-3'-nitroflavone failed to decrease flight reactions after the rat was introduced into the test area and risk assessment behavior displayed when subjects were constrained in a straight alley, and only weakly affected risk assessment of mice chased by the rat and defensive biting upon forced contact with the threat stimulus. In a drug discrimination experiment 6-bromoflavone and 6-bromo-3'-nitroflavone up to 30 and 3 mg/kg, respectively, did not substitute for the BZ chlordiazepoxide. Taken together, these results failed to demonstrate that the synthetic flavonoids 6-bromoflavone and 6-bromo-3'-nitroflavone possess

anxiolytic-like properties similar or superior to that of diazepam, as was suggested previously. Furthermore, they question the contribution of BZ-omega receptors to the behavioral effects of 6-bromoflavone and 6-bromo-3'-nitroflavone.

Hattis D. **The challenge of mechanism-based modeling in risk assessment for neurobehavioral end points.** Environ Health Perspect 1996 Apr;104 Suppl 2:381-90.
The mathematical form for a dose-time-response model is ideally not just a convenience for summarizing or fitting a particular data set--it represents a hypothesis. The more this hypothesis reflects a mechanistically sophisticated view of the likely reality, the more it can lead to potentially informative validating or invalidating types of predictions about the results of real experiments and (in the long run) reasonably credible predictions outside the range of direct observations. This paper first reviews some distinctive features of the nervous system and neurotoxic responses and theoretically explores some basic quantitative implications of these features. Relationships are derived for how dose-response relationships for the inhibition of function should depend on the numbers of neurons in series or redundant parallel arrangements that are required or capable of performing the function. Previous work is reviewed in which some less nervous-system-specific features were the foci of quantitative risk-assessment modeling for specific neurotoxic end points. These include a) rates of repair of putatively reversible damage in the case of acrylamide; b) human interindividual variability in susceptibility to fetal/developmental effects in the case of methylmercury; and c) opportunities to use intermediate biomarkers to assist in integrated animal toxicological and epidemiologic investigations of the chronic cumulative risks posed by agents that contribute to neuronal loss with increasing age and pathology.

Higginbotham SL, Ndifor MA, Reams RR. **The effects of short-term in vitro exposure of lead on neurite outgrowth and acetylcholinesterase function in a mouse neuroblastoma cell line.** Res Commun Biol Psychol Psychiat 1998;23(1-2):43-58.
BIOSIS COPYRIGHT: BIOL ABS. In this study the effects of lead acetate on neurite outgrowth, acetylcholinesterase (AChE) activity and AChE protein levels were studied in monolayer cultures of mouse neuroblastoma cells (NB41A3). The degradative enzyme of acetylcholine (Ach), AChE, was investigated in this study because it is expressed prior to neurite outgrowth. NB41A3 cells do not require nerve growth factor (NGF) to express neurites. Therefore they are a useful neuronal cell model for looking at the effect of lead (Pb) exp th at 0.5 muM Pb, a concentration considered by the CDC to be safe from increased risks of neurotoxicity for humans. Results from AChE enzyme assay and Western blot analysis indicate that Pb had no significant effect on the activity or protein level at any of the Pb concentrations tested. These findings are important because they suggest that in this cell line, low level Pb exposure (0.005-0.5 muM) have no detrimental effect, collectively, on neurite outgrowth, AChE activity and protein level.

Holownia A, Ledig M, Braszko JJ, Menez JF. **Acetaldehyde cytotoxicity in cultured rat astrocytes.** Brain Res 1999;833(2):202-8.
The effect of acetaldehyde on astrocytes have been investigated because not only do they play an important role in brain maturation but also recent reports have shown their delayed proliferation following both 'in vivo' and 'in vitro' ethanol exposure. Biochemical parameters related to apoptotic and necrotic processes were examined in primary cultures of rat astrocytes exposed for 4 days to acetaldehyde generated from ethanol by co-cultured alcohol dehydrogenase-transfected Chinese hamster

ovary cells. Acetaldehyde levels in the culture media attained concentrations of approximately 450 microM. To study ethanol effects, alcohol oxidation was inhibited by 4-methylpyrazole (an inhibitor of alcohol dehydrogenase). Acetaldehyde but not ethanol increased intracellular calcium levels by 155%. Moreover, significant DNA fragmentation was detected using a random oligonucleotide primed synthesis assay, by flow cytometry and when using agar gel electrophoresis. Transglutaminase activity was elevated in the cells treated with acetaldehyde but when acetaldehyde formation was inhibited by 4-methylpyrazole the enzyme activity was unaffected. Nitrate levels in the culture media were unchanged. Additionally, microscopic examination of cell nuclei revealed chromatin condensation in astrocytes exposed to acetaldehyde. It can be concluded, that in 'in vitro' acetaldehyde exposed rat astrocytes apoptotic pathways are activated. Copyright 1999 Published by Elsevier Science B.V.

Lamas JA. **The role of calcium in M-current inhibition by muscarinic agonists in rat sympathetic neurons.** Neuroreport 1999;10(11):2395-400.
I have investigated the role of Ca2+ on M-current (IK(M)) inhibition by the muscarinic agonist oxo-M using the perforated patch voltage clamp technique. Oxo-M inhibited IK(M) in cultured SCG cells with an IC50 of 1.2 microM in 2 mM [Ca2+]o, and 13.1 microM in nominally Ca(2+)-free external solution. BAPTA-AM, ryanodine and thapsigargin (substances which modulate [Ca2+]i) did not affect IK(M) or the inhibitory action of oxo-M in either 2 or 0 mM extracellular Ca2+. Caffeine (10 mM) inhibited M current by approximately 30% in both 2 and 0 mM [Ca2+]o; this inhibition was not affected by [Ca2+]i modulators. Unexpectedly, the effect of oxo-M (10 microM) was enhanced after application of caffeine (10 mM) in either 2 or 0 mM [Ca2+]o. Thus, the effect of muscarinic agonists on IK(M) was blunted in Ca(2+)-free extracellular solutions, but neither oxo-M nor caffeine appeared to inhibit IK(M) through an elevation of [Ca2+]i. I suggest that resting levels of [Ca2+]i are necessary for a normal inhibition, with lower levels inducing an impairment of the inhibition of IK(M) by muscarinic agonists.

Liao Y, Venhuis BJ, Rodenhuis N, Timmerman W, Wikstrom H, Meier E, Bartoszyk GD, Bottcher H, Seyfried CA, Sundell S. **New (sulfonyloxy)piperazinyldibenzazepines as potential atypical antipsychotics: chemistry and pharmacological evaluation.** J Med Chem 1999;42(12):2235-44.
A series of 2- or 8-trifluoromethylsulfonyloxy (TfO) and 2- or 8-methylsulfonyloxy (MsO) 11-piperazinyldibenzodiazepines, -oxazepines, and -thiazepines were synthesized and evaluated in pharmacological models for their potential clozapine-like properties. In receptor binding assays, the 2-TfO analogues (18a, GMC2-83; 24, GMC3-06; and previously reported GMC1-169, 9a) of the dibenzazepines have profiles comparable to that of clozapine, acting on a variety of CNS receptors except they lack M1 receptor affinity. Introduction of 2-TfO to clozapine leads to compound 9e (GMC61-39) which has a similar binding profile as that of clozapine including having M1 receptor affinity. Interestingly, the MsO analogues, as well as the 8-TfO analogues, have no or weak dopaminergic and serotonergic affinities, but all 8-sulfonyloxy analogues do have M1 affinities. In behavioral studies performed to indicate the potential antipsychotic efficacy and the propensity to induce EPS, 2-TfO analogues blocked effectively the apomorphine-induced climbing in mice in a dose-dependent manner with ED50 values (mg/kg) of 2.1 sc for 9a, 1.3 po for 18a, 2.6 sc for 24, and 8.2 sc for 9e. On the other hand, they showed a clear dose separation with regard to their ED50 values (mg/kg) for indicating catalepsy in rats (>44 sc for 9a, 28 po for 18a, 30 sc for 24, and >50 sc for 9e, respectively), thus implicating a more favorable therapeutic ratio (K/A, ED50 climbing/ED50 catalepsy)

in comparison with typical neuroleptics such as haloperidol and isoclozapine. Furthermore, compound 18a was also demonstrated to be an orally potent DA antagonist with an ED50 value of 0.7 mg/kg po in the ex vivo L-DOPA accumulation model. The present study contributes to the SAR of 11-piperazinyldibenzazepines, and the 2-TfO analogues of 11-piperazinyldibenzazepines are promising candidates as clozapine-like atypical antipsychotics with low propensity to induce EPS.

Luo FR, Wyrick SD, Chaney SG. **Comparative neurotoxicity of oxaliplatin, ormaplatin, and their biotransformation products utilizing a rat dorsal root ganglia in vitro explant culture model.** Cancer Chemother Pharmacol 1999;44(1):29-38.
PURPOSE: Neurotoxicity is one of the major toxicities of platinum-based anticancer drugs, especially oxaliplatin and ormaplatin. It has been postulated that biotransformation products are likely to be responsible for the toxicity of platinum drugs. In our preceding pharmacokinetic study, both oxaliplatin and ormaplatin were observed to produce the same types of major plasma biotransformation products. However, while the plasma concentration of ormaplatin was much lower than that of oxaliplatin at an equimolar dose, one of their common biotransformation products, Pt(dach)Cl2, was present at 29-fold higher concentrations in the plasma following the i.v. injection of ormaplatin than of oxaliplatin. Because ormaplatin has severe neurotoxicity and Pt(dach)Cl2 is very cytotoxic, we have postulated that Pt(dach)Cl2 is likely to be responsible for the differences in neurotoxicity between ormaplatin and oxaliplatin. In order to test this hypothesis, we compared the neurotoxicity of oxaliplatin, ormaplatin, and their biotransformation products. Since the dorsal root ganglia (DRGs) have been suggested to be the likely targtet for platinum drugs and in vitro DRG explant cultures have been suggested to be a valid model for studying cisplatin-associated neurotoxicity, our comparative neurotoxicity study was conducted with DRG explant cultures in vitro. METHODS: Based on the previous studies of cisplatin neurotoxicity, we established our in vitro DRG explant culture utilizing DRGs dissected from E-19 embryonic rats. Rat DRGs were incubated for 30 min with different platinum compounds to mimic in vivo exposure conditions; this was by followed by a 48-h incubation in culture medium at 37 degrees C. At the end of the incubation, the neurites were fixed and stained with toluidine blue, and neurite outgrowth was quantitated by phase-contrast microscopy. The inhibition of neurite outgrowth by platinum compounds was used as an indicator of in vitro neurotoxicity. Since an in vivo study has indicated that the order of neurotoxicity is ormaplatin > cisplatin > oxaliplatin > carboplatin as measured by morphometric changes to rat DRGs, we initially validated our DRG explant culture model by comparing the in vitro neurotoxicity of ormaplatin, cisplatin, oxaliplatin, and carboplatin. After observing the same neurotoxicity rank between this study and a previous in vivo study, we further compared the neurotoxicity of oxaliplatin, ormaplatin, and their biotransformation products including Pt (dach)Cl2, Pt(dach)(H2O)Cl, Pt(dach)(H2O)2, Pt(dach)(Met), and Pt(dach)(GSH) utilizing the DRG explant culture model. RESULTS: Our study indicated that Pt(dach)Cl2 and its hydrolysis products were more potent at inhibiting neurite outgrowth than the parent drugs oxaliplatin and ormaplatin. In contrast, no detectable inhibition of neurite outgrowth was observed for DRGs dosed with Pt(dach)(Met) and Pt (dach)(GSH). CONCLUSION: This study suggests that biotransformation products such as Pt(dach)Cl2 and its hydrolysis products are more neurotoxic than the parent drugs oxaliplatin and ormaplatin. The different neurotoxicity profiles of oxaliplatin and ormaplatin are more likely due to the different plasma concentrations of their common biotransformation product Pt(dach)Cl2 than to differences in their intrinsic neurotoxicity.

Osaka H, Mukherjee P, Aisen PS, Pasinetti GM. **Complement-derived anaphylatoxin C5a protects against glutamate-mediated neurotoxicity.** J Cell Biochem 1999;73(3):303-11.
Previous work from this laboratory indicates a role for the complement component C5 in neuroprotection against excitotoxicity. In the present study, we tested the hypothesis that the C5-derived anaphylatoxin C5a protects against kainic acid (KA)-induced neurodegeneration and investigated the mechanism of C5a neuronal activity in vitro. Brain intraventricular infusion of KA into adult mice caused neuronal morphological features of apoptosis in the pyramidal layer of the hippocampal formation as indicated by counts of neurons with pyknotic/condensed nuclei associated with cytoplasmic eosinophilia. Co-intraventricular infusion of human recombinant C5a with KA resulted in a marked reduction of morphological features of apoptotic neuronal death. In vitro studies confirmed C5a neuroprotection: treatment of primary murine corticohippocampal neurons with human or mouse recombinant C5a reduced glutamate neurotoxicity, as measured by trypan blue exclusion assay. This protection concurred with inhibition of glutamate-mediated induction of the caspase-3-related cysteine protease and coincided with marked reduction of neurons with morphological features of apoptosis, as found in vivo. Our studies indicate that C5a may inhibit glutamate-mediated neuronal death through partial inhibition of caspase-3 activity. These findings suggest a novel noninflammatory role for C5a in modulating neuronal responses to excitotoxins.

Parsons RB, Waring RH, Ramsden DB, Williams AC. **In vitro effect of the cysteine metabolites homocysteic acid, homocysteine and cysteic acid upon human neuronal cell lines.** Neurotoxicol 1998 Aug-Oct;19(4-5):599-603.
Cysteine (CYS) is a non-essential amino acid which elicits excitotoxic properties via the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. CYS levels are known to be elevated in association with neurological disease such as Alzheimers Disease (AD) and Parkinsons Disease (PD). We have previously reported studies investigating the toxicity of CYS and its major metabolite cysteinesulfinic acid (CSA) to human neuronal cell lines in vitro and in continuation of this we now report the toxicity of other compounds (Homocysteic Acid, HCA; Homocysteine, HCYS; and Cysteic Acid, CA) in the CYS metabolic pathway. Three cell lines, all of human origin and derived from separate discrete areas of the brain were used in the neurotoxicity assays. Lactate dehydrogenase (LDH) release was assayed as a measure of cell death. The cell lines investigated showed varying degrees of toxic responses which were the reverse of those seen when they were exposed to CYS or CSA. The SK. N.SH (Neuroblastoma) cell line, which exhibits a high toxic response to CYS and CSA, gave a low toxic response to HCA and CA while the TE 671 (Medulloblastoma) cell line, which exhibits a low toxic response to CYS and CSA, showed a high toxic response to HCYS, HCA and CA. However, the U-87 MG (Glioblastoma) cell line, which has a median toxic response to CYS and CSA, also has median response to HCYS, HCA and CA. These results show that toxic responses are cell-type specific for CYS and its metabolites and this may be reflected in the patterns of neurodegeneration observed in such diseases as AD and PD. HCYS is selectively toxic to medulloblastoma cells; this may explain why high HCYS levels result in neural tube defects in prenatal humans, where the same cell-type is involved.

Pittaluga A, Pattarini R, Andrioli GC, Viola C, Munari C, Raiteri M. **Activity of putative cognition enhancers in kynurenate test performed with human neocortex slices.** J Pharmacol Exp Ther 1999;290(1):423-8.

Some cognition enhancers were previously shown to potently prevent antagonism of the N-methyl-D-aspartate (NMDA)-evoked release of norepinephrine (NE) brought about in slices of rat hippocampus by kynurenic acid, an endogenous NMDA receptor blocker. We have examined the impact of putative nootropic agents in the kynurenate test performed with slices of human cerebral cortex from patients undergoing neurosurgery. In slices of human neocortex, local application of NMDA evoked release of [3H]NE; the effect of NMDA was antagonized by several NMDA receptor antagonists, including kynurenic acid. The antagonism of the NMDA-evoked [3H]NE release produced by 300 microM kynurenate was potently (EC50 <10 microM) prevented by most of the nootropics tested, including aniracetam, oxiracetam, D-cycloserine, and the glutamate analog CR 2249 (but not its enantiomer CR 2361). Nicotine or tacrine (up to 10 microM) did not show any effect in the kynurenate test. Nicotine (30-100 microM) itself increased the release of [3H]NE; interestingly, the nicotine-evoked overflow was blocked not only by the nicotin receptor antagonist mecamylamine but also by NMDA receptor antagonists, suggesting an indirect mechanism mediated by glutamate/aspartate release. To conclude, the similarities between the data obtained here with human neocortex slices and those previously obtained in the rat indicate that the kynurenate test performed with rat brain slices may represent a useful biochemical assay to study cognition-enhancing drugs.

Riikonen J, Jaatinen P, Karjala K, Rintala J, Porsti I, Wu X, Eriksson CJ, Hervonen A. **Effects of continuous versus intermittent ethanol exposure on rat sympathetic neurons.** Alcohol Clin Exp Res 1999;23(7):1245-50.
BACKGROUND: Binge ethanol exposure is known to induce degeneration of central nervous system (CNS) neurons. Sympathetic hyperactivity has been related to ethanol withdrawal symptoms, but the effects of repeated withdrawals on peripheral sympathetic neurons have not been studied previously. METHODS: The effects of continuous versus intermittent ethanol consumption on sympathetic neurons of the superior cervical ganglion (SCG) were studied in male Wistar rats. Two-month-old rats were divided into three groups: one group with ethanol (10% v/v) as the drinking fluid throughout the 51/2-month experiment (continuous, n = 9), one group drinking ethanol on 4 days/week and water on 3 days/week (intermittent, n = 9), and a control group (n = 9) with water as the only available fluid. All groups had food ad libitum. SCG volume, neuron density, and total number of neurons were measured by using unbiased morphometric methods. RESULTS: As the mean daily ethanol consumption did not differ between the two ethanol-exposed groups (continuous 5.7 g/kg/day versus intermittent 5.8 g/kg/day), the total dose of ethanol consumed was 42% smaller in the intermittent group. The total number of SCG neurons decreased by 28%, and neuron density by 23%, in the intermittent group compared with the control group, whereas no significant neuron loss was observed in the continuous group. The volume of the SCG was similar in all study groups. The results suggest that repeated ethanol withdrawals, rather than ethanol exposure per se, are deleterious to sympathetic neurons. CONCLUSIONS: Ethanol-induced degeneration of neurons is not only related to the amount of ethanol consumed, but also to the patterns of drinking.

Saunders DE, Dicerbo JA, Williams JR, Hannigan JH. **Alcohol reduces neurofilament protein levels in primary cultured hippocampal neurons.** Alcohol 1997 Sep-Oct;14(5):519-26.
High concentrations of alcohol (> or = 1.8%) were shown previously to impair health and viability of cultured hippocampal neurons. Because neurofilament proteins are essential for neuronal process

outgrowth and differentiation, the effects of alcohol on these proteins were determined in the neuronal processes of primary cultured gestational day 18 rat hippocampal neurons. At the relatively lower concentrations used in the present study, alcohol caused a concentration-dependent reduction (< or = 47%) in 68 and 200 kDa neurofilament proteins ($p < 0.05$). Alcohol caused a 32% downward trend in 160 kDa neurofilament protein levels. Alcohol up to 1% (72-h exposure) produced no obvious alterations in neurite extension or explant morphology, and there were no visual signs of cell death. The sensitive MTT dye reduction assay showed no biochemical evidence of decreased cell viability at < or = 0.5% alcohol. The 32-47% reductions in neurofilament protein levels in vitro may hold implications for later hippocampal neuronal differentiation events in animals prenatally exposed to alcohol.

Sawyer TW. **Toxicity of sulfur mustard in primary neuron culture.** Toxicol In Vitro 1999;13(2):249-58.
BIOSIS COPYRIGHT: BIOL ABS. The toxicity of sulfur mustard (HD) was assessed in primary cultures of chick embryo forebrain neurons using several different endpoints. Mature neurons were found to be very sensitive to the toxic effects of this agent and tritiated arachidonic acid release, as well as the MTT, neutral red and alamarBlue cytotoxicity assays all gave LC50 values in the low muM range. Maximal toxicity was initiated within minutes of culture exposure to HD and was not found to be associated with toxic mediator rel at the time of chemical treatment. Although the reasons for these observations are unclear, theapparent dependence of HD toxicity on the differentiative maturity of the cultures may eventually provide some clues as to the mechanism of action of this chemical agent. Furthermore, the extreme sensitivity of these cells to the toxic effects of HD makes them a useful model system with which to screen for potential protective drug regimens against this chemical warfare agent.

Shinomura T, Nakao S, Adachi T, Shingu K. **Clonidine inhibits and phorbol acetate activates glutamate release from rat spinal synaptoneurosomes.** Anesth Analg 1999;88(6):1401-5.
Glutamate is a major neural transmitter of noxious stimulation in the spinal cord. We measured glutamate release from rat spinal synaptoneurosomes by using an enzyme-linked fluorimetric assay. Glutamate was released from spinal cord synaptoneurosomes in response to the addition of 30 mM potassium chloride, 1 mM 4-aminopyridine, or 1 microM ionomycin in the presence of external calcium. There was less release of glutamate in the absence, versus the presence, of external calcium. Clonidine significantly reduced the level of glutamate released from the spinal cord synaptoneurosomes. Tetradecanoyl phorbol acetate, an activator of protein kinase C, enhanced glutamate release. Forskolin, a protein kinase A activator, had no effect on the glutamate efflux. Our data indicate that glutamate released in the spinal cord is dependent on protein kinase C but is independent of the protein kinase A pathway. They also suggest that the inhibition of glutamate release may be the underlying mechanism of antinociception by clonidine at the spinal cord level. IMPLICATIONS: We demonstrated that synaptoneurosomes from rat spinal cord could release glutamate in response to depolarization. We showed that an activator of protein kinase C increased glutamate released from spinal cord synaptoneurosomes but that clonidine decreased it. Glutamate release may be one of the mechanisms of antinociception at the spinal cord level.

Strahlendorf JC, Strahlendorf HK. **Enduring changes in Purkinje cell electrophysiology following transient exposure to AMPA: correlates to dark cell degeneration.** Neurosci Res 1999;33(2):155-62.
Purkinje cells (PCs) are selectively vulnerable to alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic

acid (AMPA)-mediated delayed toxicity that is manifested as dark cell degeneration (DCD) rather than necrosis. The purpose of the present study was to utilize electrophysiologic changes induced by AMPA to gain mechanistic insights into its cytotoxic actions. The whole-cell configuration of the patch clamp technique was used to record spontaneous electrical activity and ionic currents of Purkinje neurons from cerebellar slices using an experimental paradigm known to produce DCD in response to AMPA. Initial electrophysiologic responses to AMPA consisted of a large transient depolarization and inward current that declined by 75% 20 min into the 30-min exposure to 30 microM AMPA. Cellular responses temporarily continued towards basal levels following removal of AMPA. A sustained membrane depolarization (and underlying persistent inward current), an abundance of apparent excitatory synaptic events, and loss of electro- and chemoresponsiveness were observed 60-75 min into the expression phase (following AMPA removal). These events correspond temporally to the development of DCD in Purkinje cells and may represent an electrophysiological signature of AMPA receptor-mediated delayed neurotoxic events. Antagonists of the AMPA receptor present concomitantly with AMPA are known not to affect DCD and failed to alter the electrophysiologic changes. The secondary depolarization and loss of electroresponsiveness were prevented by antagonists present after removal of AMPA, at a time when DCD also is prevented. Electrical clamping of the PC membrane to equivalent depolarized membrane potentials (V(m)s) obtained with AMPA failed to elicit any long lasting alterations in PC physiology. Collectively, morphological and electrophysiological data indicate that induction of DCD is not strongly dependent on ionotropic mechanisms elicited by AMPA receptors, but that expression of DCD does possess an ionotropic element.

Wictome M, Newton KA, Jameson K, Dunnigan P, Clarke S, Gaze J, Tauk A, Foster KA, Shone CC. **Development of in vitro assays for the detection of botulinum toxins in foods.** FEMS Immunol Med Microbiol 1999;24(3):319-23.
Currently the only accepted method for the detection of botulinum neurotoxin in contaminated samples is the mouse bioassay. Although highly sensitive this test has a number of drawbacks: it is expensive to perform, lacks specificity and involves the use of animals. With increasing resistance to such animal tests there is a need to replace the bioassay with a reliable in vitro test. Over the past six years it has been demonstrated that all the botulinum neurotoxins act intracellularly as highly specific zinc endoproteases, cleaving proteins involved in the control of secretion of neurotransmitters. In the work described, this enzymatic activity has been utilised in assay formats for the detection in foods of neurotoxin of the serotypes involved in food-borne outbreaks in man. These assays have been shown to have a greater sensitivity, speed and specificity than the mouse bioassay. It is envisaged that such assays will prove realistic alternatives to animal-based tests.

Wictome M, Newton K, Jameson K, Hallis B, Dunnigan P, Mackay E, Clarke S, Taylor R, Gaze J, Foster K, et al. **Development of an in vitro bioassay for Clostridium botulinum type B neurotoxin in foods that is more sensitive than the mouse bioassay.** Appl Environ Microbiol 1999;65(9):3787-92.
BIOSIS COPYRIGHT: BIOL ABS. A novel, in vitro bioassay for detection of the botulinum type B neurotoxin in a range of media was developed. The assay is amplified by the enzymic activity of the neurotoxin's light chain and includes the following three stages: first, a small, monoclonal antibody-based immunoaffinity column captures the toxin; second, a peptide substrate is cleaved by using the endopeptidase activity of the type B neurotoxin; and finally, a modified enzyme-linked immunoassay

system detects the peptide cleavag l antibodies as the capture phase, we found that the endopeptidase assay was capable of differentiating between the type B neurotoxins produced by proteolytic and nonproteolytic strains of Clostridium botulinum type B.

Zheng JQ, He XP, Yang AZ, Liu CG. **Physostigmine blocked nicotinic acetylcholine receptors in rat sympathetic ganglion neurons.** Chung Kuo Yao Li Hsueh Pao 1997;18(6):508-11.
AIM: To study the blocking mechanism of physostigmine (Phy) on nicotinic acetylcholine receptors (NAChR) in sympathetic neurons. METHODS: The whole-cell patch-clamp technique was used to observe the effects of Phy on NAChR in the cultured sympathetic neurons from neonatal rat superior cervical ganglia (SCG). RESULTS: Phy 5 -20 mumol.L-1 inhibited neuronal NAChR in a concentration-dependent manner and accelerated the desensitization of NAChR. Changing the membrane potential from -50 to -90 mV did not affect the blocking effect of Phy. Phy 200 mumol.L-1 did not induce any noticeable response in SCG neurons. CONCLUSION: Phy blocked NAChR in the sympathetic ganglion neurons by interacting with the allosteric sites out of the binding sites and the open ionic channels of the receptors. Phy did not possess excitative effect on NAChR in SCG neurons.

OCULAR TOXICITY

Angelini C, Costa M, Morescalchi F, Cimoli G, Coniglio L, Falugi C. **Muscarinic drugs affect cholinesterase activity and development of eye structures during early chick development.** Eur J Histochem 1998;42(4):309-20.
During neurogenesis, markers of the cholinergic system are present in the eye and visual cortex of vertebrates. In adult vertebrates, a role for these molecules, including muscarinic acetylcholine receptors (mAChRs), in eye growth non-accommodative regulation is also known. In order to understand the biological mechanisms triggered by the cholinergic system in these events, we analysed the effects of a cholinergic agonist (10(-4) M carbachol) and an antagonist (10(-4) M atropine) of the muscarinic receptors, on early chick development. To establish if the cholinergic system also plays a role in the regulation of early neurogenetic signals, the drug treatments were made at stage 5-6 HH, during the formation of the cephalic process. Specific effects on forehead, and in particular on eye development were found; carbachol treated embryos presented huge and well pigmented eyes, significantly different from controls. The eyes of atropine-exposed embryos presented anomalies with different phenotypes ranging from strongly affected features to normal-like appearance. Generally, the eyes were smaller as compared to the controls, with a number of anomalies, also in the normal-like phenotype, including retina and lens defects. In these structures, distribution of cholinesterase activities was checked by histochemical methods, and the amount of cells undergoing nuclear disgregation was revealed by DAPI staining. We propose that the drugs affected the known nervous and pre-nervous functions of the cholinergic markers, such as cell signalling during primary induction, and regulation of cell death by ACh receptors.

Bagley DM, Cerven D, Harbell J. **Assessment of the chorioallantoic membrane vascular assay (CAMVA) in the COLIPA in vitro eye irritation validation study.** Toxicol In Vitro 1999;13(2):285-93.
BIOSIS COPYRIGHT: BIOL ABS. The chorioallantoic membrane vascular assay (CAMVA) is an

alternative to the Draize rabbit eye irritation method. The CAMVA employs the vascularized membrane of a fertile hen's egg to assess eye irritation potential. This irritation potential is a function of alterations in the vasculature following the administration of test material. Because of the history of use of the CAMVA it was selected as one of the methods for a validation study organized and sponsored by COLIPA. For this validation stu sets of data were compared to assess the interlaboratory reproducibility of the assay. The results of this validation study of the CAMVA show that for test materials with MMASs in the 0 to 5 range or the 55 to 110 range, the CAMVA did not give a good prediction. The predictions were better for samples of mild to moderate irritation (MMAS 5-55). The difficulty in predicting at the low end of the irritation scale appears to be due to the biological variability of the test system and the subjective by reducing the subjectivity in the scoring and (2) to develop better prediction models using more data in the range of severe irritants.

Braunstein SG, Deramaudt TG, Rosenblum DG, Dunn MG, Abraham NG. **Heme oxygenase-1 gene expression as a stress index to ocular irritation.** Curr Eye Res 1999;19(2):115-22.
PURPOSE. Predicting the toxic potential of compounds to the ocular surface has depended on the Draize test for the past half century. Alternatives to Draize testing have recently been sought for a number of reasons. Stress gene expression has emerged as a means of quantifying cellular reaction and, thus, the toxic potential of the compound in question. This study examines the expression of the major stress response gene heme oxygenase-1 (HO-1) in a human corneal epithelial cell line (HCE-T) following challenges with a number of known ocular irritants. METHODS. HCE-T was used to investigate the effect of ocular irritants on cell viability and HO-1 expression. Irritants tested included hydrogen peroxide, isopropyl alcohol, sodium hydroxide and trichloroacetic acid. HCE-T cells were grown to 80% confluency and treated with the listed irritants at a concentration range of 10-100 microM. Cell viability and northern blot analysis were performed following a 24 and 48 hr incubation period. RESULTS. HCE-T cells expressed HO-1 mRNA and HO activity similar to other human cell lines. Northern blot analysis demonstrated that levels of HO-1 mRNA transcripts increased regularly after exposure to the irritants in a concentration-dependent manner. Studies on the effect of various inhibitors and inducers of HO-1 on cell viability showed that inhibition of HO-1 potentiates the cytotoxic effect of ocular irritants. In contrast, pre-induction of HO-1 in HCE-T decreases the effect of various irritants on cell viability. CONCLUSIONS. These results are consistent with the idea that HO-1 mRNA levels may be used as an indicator of toxicity resulting from ocular irritants and that HCE-T cells respond to stress in a fashion similar to other human cell lines. This strategy for testing may be important in the development of an alternative to Draize testing. The results of this study also suggest that HO-1 may constitute a part of the protective defense mechanism against chemical injury.

Courtellemont P, Hebert P, Biesse JP, Castelli D, Friteau L, Serrano J, Robles C. **Relevance and reliability of the PREDISAFE assay in the COLIPA eye irritation validation program (Phase 1).** Toxicol In Vitro 1999;13(2):305-12.
BIOSIS COPYRIGHT: BIOL ABS. The 6th Amendment of the European Directive on Cosmetics induces a potential ban on animal testing for cosmetic ingredients and finished products. In this new context, COLIPA (The European Cosmetic Toiletry and Perfumery Association) has initiated an international multicentric study with the main goal of validating available alternatives to in vitro methods for assessing the eye irritation potential of cosmetic raw materials and formulations. In order to

test undiluted and hydrophobic ingredient ritation program are described, and furthermore the PREDISAFE assay results obtained during Phase Iof the above mentioned study are presented and discussed in detail. The statistical analysis proves clearly a great interest in the PREDISAFE test for the prediction of eye irritation potential of cosmetic formulations. Its strong compatibility for a wide category of finished products associated with its ease of use offer relevant advantages for a routine use in the ocular irritancy screening in th o assays for acceptance by the regulators for the replacement of the Draize eye irritation test.

Courtellemont P, Pannetier M, Biesse JP, Larnicol M, Baret JP, Breda B. **Evaluation of the EYTEX system in the COLIPA eye irritation program.** Toxicol In Vitro 1999;13(2):295-304.
BIOSIS COPYRIGHT: BIOL ABS. In the context of the 6th Amendment of the European Directive on Cosmetics, several cosmetic companies concentrate their basic research on the development of the best adapted battery of in vitro tests able to be incorporated in the ocular risk assessment process. Consequently, the European Cosmetic Toiletry and Perfumery Association (COLIPA) has initiated an international multicentric study with the main purpose to validate available alternatives in vitro methods for assessing the eye irritation and the different protocols used in the COLIPA study are described. Then, the EYTEX assay results are presented and discussed in details in order to understand the failure of this assay during this validation study. The relevance and the reliability of the EYTEX assay were particularly low in two laboratories, whereas one laboratory presented acceptable data with a low compatibility with tested samples. These results underline the problem of the complex qualification process of this assay, since er definition of limitations of the EYTEX assay seems to be necessary to better harmonize the qualification procedure in the three laboratories. The COLIPA validation process clearly demonstrated that the EYTEX assay was first, not suitable for the assessment of the eye irritation potential of surfactants and formulations based on surfactants, and secondly not ready for a validation study requiring the establishment of adequate and well defined mathematical prediction models. However, internal c.

Doucet O, Lanvin M, Zastrow L. **Comparison of three in vitro methods for the assessment of the eye-irritating potential of formulated cosmetic products.** In Vitro Mol Toxicol 1999;12(2):63-76.
BIOSIS COPYRIGHT: BIOL ABS. This study aimed at comparing the potential of three alternative methods with the Draize eye irritation test. A cytotoxicity assay achieved by using a new commercialized reconstituted human epithelial culture model (REC), and two widely used in vitro methods: the Het-Cam and the neutral red uptake cytotoxicity assay (NRU) were performed for predicting the eye-irritating potential of formulated cosmetic products. Ten skin-care products, ten sunscreen products, ten surfactant-based products, and t learly overestimated. More dramatically, the NRU assay failed to identify 19 products, ten of them being underestimated. The linear correlation established between the in vivo and in vitro endpoints led to similar conclusions. The only statistically relevant correlation was obtained by plotting REC assay data and Draize scores. Neither the Het-Cam nor the NRU assay scores satisfactorily correlated with the Draize values. We concluded that this simple cytotoxicity test, achieved with a reconstitu.

Gadea A, Lopez E, Lopez-Colome AM. **Characterization of glycine transport in cultured Muller glial cells from the retina.** Glia 1999;26(4):273-9.
Rapid termination of the synaptic action of glutamate (Glu) and glycine (Gly) is achieved by uptake into

the presynaptic terminal and glial cells. In the vertebrate CNS, Gly acts both as an inhibitory neurotransmitter and as a Glu modulator or coagonist at postsynaptic N-methyl-D-aspartate (NMDA) receptors. We have previously described NMDA receptors in Muller cells of chick retina coupled to the phosphoinositide cascade, the entry of calcium, and the activation of protein kinase C (PKC; Lopez-Colome et al. Glia 9:127-135, 1993). A colocalization of Gly transporters and NMDA receptors has been reported in brain tissue (Smith et al. Neuron 8:927-936, 1992); since the concentration of Gly could participate in the modulation of Glu excitatory transmission in the vertical pathways of the retina, transport of Gly in monolayer cultures of Muller cells was studied. Gly transport was found pH-sensitive with an optimum at pH 7.4. Kinetic analysis of the saturation curve for Gly within a concentration range of 0.01-2 mM, revealed two components of transport: a low-affinity system with Km = 1.7 mM, Vmax = 30 nmol/10 min/mg protein, and a high-affinity one with a Km = 27 microM, Vmax = 3 nmol/10 min/mg protein. Both systems were Na+ -dependent; the high-affinity system proved also dependent on external Cl- and was inhibited by sarcosine, characteristic of GLYT1 transporters. The inhibition of low-affinity uptake by 2-(methylamino)isobutyric acid (MeAIB) and 2-aminoisobutyric acid (AIB) suggests the presence of transport system A in Muller cells. The process is energy-requiring, since Gly transport was decreased by metabolic inhibitors. Data obtained are in keeping with a modulatory role for Muller glia on excitatory transmission in the retina.

Germain L, Auger FA, Grandbois E, Guignard R, Giasson M, Boisjoly H, Guerin SL. **Reconstructed human cornea produced in vitro by tissue engineering.** Pathobiology 1999;67(3):140-7.
The aim of the present study was to produce a reconstructed human cornea in vitro by tissue engineering and to characterize the expression of integrins and basement membrane proteins in this reconstructed cornea. Epithelial cells and fibroblasts were isolated from human corneas (limbus or centre) and cultured on plastic substrates in vitro. Reconstructed human corneas were obtained by culturing epithelial cells on collagen gels containing fibroblasts. Histological (Masson's trichrome staining) and immunohistological (laminin, type VII collagen, fibronectin as well as beta1, alpha3, alpha4, alpha5, and alpha6 integrin subunits) studies were performed. Human corneal epithelial cells from the limbus yielded colonies of small fast-growing cells when cultured on plastic substrates. They could be subcultured for several passages in contrast to central corneal cells. In reconstructed cornea, the epithelium had 4-5 cell layers by the third day of culture; basal cells were cuboidal. The basement membrane components were already detected after 3 days of culture. Integrin stainings, except for the alpha4 integrin, were also positive after 3 days. They were mostly detected at the epithelium-stroma junction. Such in vitro tissue-engineered human cornea, which shows appropriate histology and expression of basement membrane components and integrins, provides tools for further physiological, toxicological and pharmacological studies as well as being an attractive model for gene expression studies.

Grosskreutz CL, Katowitz WR, Freeman EE, Dreyer EB. **Lidocaine toxicity to rat retinal ganglion cells.** Curr Eye Res 1999;18(5):363-7.
PURPOSE: To examine the effects of the local anesthetic, lidocaine, on rat retinal ganglion cells (RGC) in vitro and in a modified in vivo assay. METHODS: For in vitro experiments, RGC were dissociated from freshly harvested Long Evan's rat pup retinas. The RGC were incubated overnight with varying concentrations of lidocaine (0.5-12.0 mM). Surviving cells were assayed at 24 hours. In an in vivo assay, 7-day-old Long-Evans rat pups were anesthetized and 2 microl of lidocaine (final intraocular

concentration: 0.03-15 mM) or vehicle was injected intravitreally. Intravitreal coinjection of nimodipine or MK801 (dizocilpine) were also performed in a subset of animals. A week after injection, rat pups were sacrificed and each retina removed, dissociated and plated separately. RGC survival was immediately assessed. Living RGC were identified on the basis of morphology and counted in a masked fashion. RESULTS: Lidocaine is toxic in a dose dependent fashion to RGC in vitro. Lower concentrations (0.5 mM and 1.0 mM) were non-toxic; 2.0, 6.0 and 12.0 mM lidocaine killed 25%, 88% and 99% of the RGC respectively. Intravitreal lidocaine was also toxic to RGC in a dose dependent fashion. Lidocaine concentrations of 3.0 mM, 7.5 mM and 15 mM killed 25%, 38% and 44% of the RGC. This effect was blocked by the simultaneous administration of either nimodipine or MK801. CONCLUSIONS: Lidocaine is toxic to RGC both in vitro and in vivo. This effect is blocked in vivo by the simultaneous administration of agents known to block glutamate mediated neuronal death, suggesting that excitotoxicity may be involved in this process.

Hagino S, Kinoshita S, Tani N, Nakamura T, Ono N, Konishi K, Iimura H, Kojima H, Ohno Y. **Interlaboratory validation of in vitro eye irritation tests for cosmetic ingredients. (2) Chorioallantoic membrane (CAM) test.** Toxicol In Vitro 1999;13(1):99-113.
BIOSIS COPYRIGHT: BIOL ABS. A chorioallantoic membrane (CAM) assay evaluates the blood vessel reaction and damage to the CAM of a fertilized hen's egg. Two types of CAM assays, the hen's egg test-chorioallantoic membrane (HET-CAM) method and the chorioallantoic membrane-trypan blue staining (CAM-TB) method, were evaluated as alternative methods to the Draize eye irritation test (Draize test). The validation project was composed of three test phases in which 10, 15 and 14 test chemicals, respectively, were evaluated. The te copic observation, suggesting that the objectivity and quantitativeness differs between the assay systems. The average values using these two methods were compared with the maximum average Draize total score (MAS). The correlation coefficient (r) between the HET-CAM scores and the MAS was 0.688. This suggests that a simple linear regression may not be appropriate for HET-CAM. However, the Spearman's rank correlation coefficient (rs) was relatively high (rs = 0.802). In contrast, the CAM-TB test.

Harbell JW, Osborne R, Carr GJ, Peterson A. **Assessment of the Cytosensor microphysiometer assay in the COLIPA in vitro eye irritation validation study.** Toxicol In Vitro 1999;13(2):313-23.
BIOSIS COPYRIGHT: BIOL ABS. The Cytosensor microphysiometer assay and its associated prediction model were evaluated in the COLIPA ocular irritation validation study for cosmetic ingredients and formulations. Test materials were prepared in low-buffer medium and exposed to L929 cells grown in transwells. The metabolic rate of the cell population was measured after each dose and the dose inducing a 50% decrease in the rate (MRD50) was determined and used to predict the ocular irritation potential. Only 29 of the 55 materia nt interlaboratory reproducibility for the MRD50 values across all test materials was observed.

Hatao M, Murakami N, Sakamoto K, Ohnuma M, Matsushige C, Kakishima H, Ogawa T, Kojima H, Matsukawa K, Masuda K, et al. **Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (4) Haemoglobin denaturation test.** Toxicol In Vitro 1999;13(1):125-37.
BIOSIS COPYRIGHT: BIOL ABS. Interlaboratory validation of the haemoglobin denaturation (HD) test on 38 cosmetic ingredients was conducted by five to eight participating laboratories. The HD test was evaluated as an alternative method to the Draize eye irritation test (Draize test) based on three

indices of protein denaturation: the test substance concentration that induces 50% HD of the positive control (RDC50), a relative HD rate at 1% of the test substance (1%RDR) and a relative change in maximum absorption wavelength (1 0.79, respectively. The results revealed several limitations associated with the HD test: (1) the HD test cannot be applied to coloured test substances with a strong absorption, around 418 nm; (2) water-insoluble test substances cannot be evaluated by RDC50 or 1% RDR; (3) the HD test cannot be applied to strong acids that exceed the buffering capacity of a phosphate buffer solution; (4) the HD test cannot be used to determine the potential for eye irritation caused by factors other than protein.

Jones PA, Bracher M, Marenus K, Kojima H. **Performance of the neutral red uptake assay in the COLIPA international validation study on alternatives to the rabbit eye irritation test.** Toxicol In Vitro 1999;13(2):325-33.
BIOSIS COPYRIGHT: BIOL ABS. The neutral red uptake (NRU) assay was included as part of the COLIPA international validation trial of in vitro alternatives to the Draize eye irritation test. In a blind trial, 55 substances were tested at four laboratories. Following testing, a prediction of the in vivo Draize modified maximum average score (MMAS) for each substance was made by each laboratory using a prediction model relating mean NR50 value (concentration causing 50% reduction in NRU from that of untreated control cells) to ween observed and predicted MMAS (using the proposed prediction model) when all the test substances were analysed together (r = 0.246). Data analysis of subsets of substances indicated that the best predictions were for pure surfactants only (r = 0.843) although this data did not fit within the limits of the prediction model. The NRU assay therefore appears to have limited use as a complete Draize replacement. A further examination of the COLIPA trial data may identify combinations of assays whi.

Joo C, Cho K, Kim H, Choi JS, Oh YJ. **Protective role for bcl-2 in experimentally induced cell death of bovine corneal endothelial cells.** Ophthalmic Res 1999;31(4):287-96.
To characterize the pattern of cell death and to investigate the potential role of bcl-2 in a death paradigm of corneal endothelial cells, primary cultures of bovine corneal endothelial (BCEN) cells were first established and treated with 0.01-1 microM staurosporine, a nonspecific protein kinase inhibitor. The pattern of BCEN cell death induced by staurosporine was apoptotic in nature, characterized by shrinkage of the cytoplasmic membrane, nuclear condensation and DNA fragmentation. Cotreatment of BCEN cells with Z-VAD-fmk (a caspase inhibitor) but not cycloheximide (a protein synthesis inhibitor) prevented staurosporine-induced cell death. To investigate the potential role of bcl-2, BCEN cells were transferred with a eukaryotic expression vector containing anti-apoptotic bcl-2 cDNA and characterized by reverse transcription-polymerase chain reaction (RT-PCR; BCEN/bcl-2). As measured by the MTT reduction assay after treatment with staurosporine, the survival rate of BCEN/bcl-2 cells was 48.0 +/- 4.8% compared to 7.4 +/- 2.1% in control BCEN cells. As determined by light microscopy, apoptotic changes such as nuclear condensation and apoptotic bodies were largely attenuated in BCEN/bcl-2 cells after staurosporine treatment although arborization of processes and rounding up of the cell body were not affected by overexpression of bcl-2. These results suggest that staurosporine induces apoptosis in a cycloheximide-independent but caspase-dependent manner and bcl-2 acts as a negative regulator in staurosporine-induced apoptosis of BCEN cells.

Kristen U, Jung K, Pape W, Pfannenbecker U, Rensch A, Schell R. **Performance of the pollen tube**

growth test in the COLIPA validation study on alternatives to the rabbit eye irritation test. Toxicol In Vitro 1999;13(2):335-42.
BIOSIS COPYRIGHT: BIOL ABS. In the present paper, we describe and analyse the performance and the results of the pollen tube growth (PTG) test applied to the COLIPA international validation study of in vitro alternatives to the Draize eye irritation test. The PTG test, based on photometric quantification of in vitro pollen tube mass production, was used by three independent laboratories to estimate the acute eye irritation potentials of 23 ingredients and 32 cosmetic formulations. Basing on historical Draize test data and nic surfactants, and acidic and alkaline materials. Furthermore, our results indicated that the PTG test was able to produce precise IC50 values without any limitations from all of the 55 test substances with good intra- and interlaboratory reproducibility. From these findings we suggest that the PTG test is not a validated test at present but is considered to be a potent candidate for further validation processes. For this purpose an additional prediction model for ingredient classes as mention.

Kulkarni AS, Hopfinger AJ. **Membrane-interaction QSAR analysis: Application to the estimation of eye irritation by organic compounds.** Pharm Res 1999;16(8):1245-53.
BIOSIS COPYRIGHT: BIOL ABS. Purpose. The purpose of this study was to explore a potential mechanism of eye irritation, and to construct a corresponding general quantitative structure-activity relationship (QSAR) model, in terms of diversity of irritant chemical structure, based on the Draize eye irritation ECETOC data set. Methods. Molecular dynamic simulation (MDS) was used to generate intermolecular membrane-solute interaction properties. These intermolecular properties were combined with intramolecular physicochemical p sicochemical descriptors were selected from a trial set of 95 descriptors for 18 structurally diverse compounds fully representative of the ECETOC set of 38 compounds. Conclusions. Combining intermolecular solute-membrane interaction descriptors with intramolecular solute descriptors yields statistically significant eye irritation QSAR models. The resultant QSAR models support an eye irritation mechanism of the action in which increased aqueous solubility of the irritant and its strength of bind.

Lindfors NC, Klockars M, Ylanen H. **Bioactive glasses induce chemiluminescence by human polymorphonuclear leukocytes.** J Biomed Mater Res 1999;47(1):91-4.
The effect of bioactive glasses on human polymorphonuclear leukocytes (PMNLs) were studied in vitro by a chemiluminescence (CL) assay. Eight different glasses were chosen. All glasses induced a rapid CL response by human PMNLs, which proved to be dose dependent. The CL response also seemed to depend on the durability of the glasses. The least durable glass caused the highest CL response, and highly durable glasses caused only low CL responses by the cells. Copyright 1999 John Wiley & Sons, Inc.

Linetsky M, James HL, Ortwerth BJ. **Spontaneous generation of superoxide anion by human lens proteins and by calf lens proteins ascorbylated in vitro.** Exp Eye Res 1999;69(2):239-48.
The proteins isolated from aged human lenses and brunescent cataracts exhibit extensive disulfide bond formation. Diabetic rat lenses similarly contain disulfide-bonded protein aggregates. These observations are consistent with the known link between diabetes, glycation and oxidative damage, and suggest a role for reactive oxygen species (ROS) in this process. To assess whether the glycation-related modifications in human lens proteins spontaneously generate ROS, superoxide anion formation was measured using both cataractous lens proteins and calf lens proteins glycated in vitro with ascorbic acid (ascorbylated).

The water-insoluble fraction from aged normal human lenses generated 0.3-0.6 nmol superoxide h(-1) mg protein(-1), whereas the activity increased to 0.5-1.8 nmol h(-1)mg protein(-1)with the WI fraction from brunescent cataracts, and 2.3 nmol h(-1)mg protein(-1)with calf lens proteins ascorbylated for 4 weeks in vitro. The activity in the human lens proteins was observed in both the water-soluble and water-insoluble fractions, and was completely dependent upon the presence of oxygen. The pH optimum curve for superoxide formation increased from pH 6.5 to 10 with both the cataract and ascorbylated proteins. The superoxide-generating activity in human lens was completely bound to a boronate affinity column, but only partially bound with the ascorbylated proteins.The superoxide anion produced by a 5 m m solution of purified N(epsilon)-fructosyl-lysine was barely detectable, and therefore, could not account for the superoxide formed by any of the lens protein preparations. Also, superoxide formation increased 10-fold at pH 8.8 with fructosyl-lysine, but only 1.3-1.8-fold with human lens proteins. The addition of copper-stimulated superoxide formation with glycated bovine serum albumin, but no stimulation was seen with cataractous proteins. Assays of specific compounds showed that catechol, hydroquinone, 3-OH kynurenine and 3-OH anthranylic acid exhibited the greatest activity for superoxide generation, but had a very short halflife. 2,3-Dihydroxypyridine and 4,5 dihydroxynaphthalene were one and two orders of magnitude less reactive.In long-term incubations at 37 degrees, cataractous proteins retained the potential to produce superoxide anion, losing only half of the initial activity after 6-7 days. Therefore, the water-insoluble fraction from aged human lenses and dark brown cataracts are potentially capable of generating >100 nmol mg protein(-1)and >170 nmol mg protein(-1)of superoxide anion respectively, likely due to the presence of advanced glycation endproducts in human lens proteins. This spontaneous generation of superoxide anion in vivo could account for a major portion of the oxidation of sulfur amino acids seen during aging and cataract formation. Copyright 1999 Academic Press.

Lordo RA, Feder PI, Gettings SD. **Comparing and evaluating alternative (in vitro) tests on their ability to predict the draize maximum average score.** Toxicol In Vitro 1999;13(1):45-72. BIOSIS COPYRIGHT: BIOL ABS. The Cosmetic, Toiletry, and Fragrance Association (CTFA) Evaluation of Alternatives Program comprised a multi-phased study of the relationship between Draize eye irritation test data and comparable data from a selection of promising alternative (in vitro) tests. The CTFA Program was designed to determine the effectiveness and limitations of several in vitro tests over a range of different cosmetic and personal-care product types. Test materials constituted experimental formulations representativ to warrant further statistical analysis. In vitro test performance was then evaluated by regression modelling of these relationships. Maximum average Draize score (MAS) was utilized as the primary quantitative measure of eye irritation potential in vivo. The goodness-of-fit of the observed data to the regression model and comparison of the magnitude of upper and lower prediction-bounds on the range of probable MAS values associated with the regression model fit (prediction intervals) provide a m hases I and II, the widths of the prediction intervals were narrowest in the region corresponding to low irritation potential; increasing widths were observed as irritation potential increased. In Phase III, relatively narrow prediction interval widths were observed at both the low and high end of the observed range of irritation potential; wider intervals were observed in the middle of the observed range. In general, the selected endpoints in each phase had similar average prediction interval w d by the model for a given formulation. Consistently, this component is responsible for 70% to 95% of the total variability. The other components (i.e. variability among replicate MAS and in vitro scores) could be reduced simply by increasing the number of replicate tests performed on each test formulation.

However, this would have relatively little impact on the overall precision of prediction.

Lovell DP. **Principal component analysis of tissue scores from substances used in the COLIPA Eye Irritation Validation Study.** Toxicol In Vitro 1999;13(3):491-503.
BIOSIS COPYRIGHT: BIOL ABS. Principal component analyses (PCA) have been carried out on the tissue scores from Draize eye irritation tests on the 55 formulations and chemical ingredients included in the COLIPA Eye Irritation Validation Study. A PCA was carried out on the tissue scores 24, 48 and 72 hours after instillation of the substances. The first Principal Component (PC I) explained 77% of the total variation in the tissues scores and showed a high negative correlation (r = -0.971) with the scores used to derive the M corneal opacity but large corneal area scores. This may represent some particular manner of scoringat the laboratory administering the Draize test or a specific effect of some formulations. A further PCA was carried out on tissue scores from observations at 1 hr to 21 days. PC I in this analysis explained 62% of the variability and there was a high negative correlation with the sum of all the tissue scores, while PC II explained 14% of the variability and contrasted damage up to 72 hours with d kely to be of any advantage in using individual tissue scores for comparisons with alternative tests. The relationship of the classifications schemes used by three alternative methods in the COLIPA study with the results of the PCA were investigated and the implications of the effect of persistence of tissue damage for various classifications schemes visualized.

Matsukawa K, Masuda K, Kakishima H, Suzuki K, Nakagawa Y, Matsushige C, Imanishi Y, Nakamura T, Mizutani A, Watanabe R, et al. **Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (11) EYTEXTM.** Toxicol In Vitro 1999;13(1):209-17.

Maurer JK, Jester JV. **Use of in vivo confocal microscopy to understand the pathology of accidental ocular irritation.** Toxicol Pathol 1999;27(1):44-7.
In vivo confocal microscopy (CM) provides a unique ability to section optically through living, intact tissues and organs to characterize qualitatively and quantitatively pathological changes in 4 dimensions (x, y, and z, and time). It involves the capture of real-time images without the need for excision, fixation and processing. In vivo CM principally has been used for evaluation of eyes in patients and laboratory animals but has potential application to studies of other tissues/organs. In vivo CM is being used in human ophthalmology clinics. It has been used as a research tool for quantitative, in situ measurement of corneal wound contraction, fibroblast migration, corneal endothelial cell migration, corneal epithelial cell size and desquamation following contact lens wear and surgery, and the assessment of corneal surface toxicity following application of commonly used ophthalmic preservatives. In vivo CM allows us to (a) characterize changes to a light microscopic (i.e., cellular) level; (b) quantify changes objectively: (c) conduct studies of injury and repair in the same animal and directly correlate microscopic changes to clinical observations over time as this technique is used in the living animal; and (d) conduct comparative studies in humans. Here we present a brief overview of in vivo CM and how we are using it to provide noninvasive, in situ qualitative and quantitative histopathologic characterization of accidental ocular irritation. Our intent is to provide an awareness of this relatively new methodology and one practical application of its use in research. The goal of our work is to provide objective, quantitative data for use in developing and validating mechanistically based in vitro replacement tests.

Maurer JK, Parker RD, Petroll WM, Carr GJ, Cavanagh HD, Jester JV. **Quantitative measurement of**

acute corneal injury in rabbits with surfactants of different type and irritancy. Toxicol Appl Pharmacol 1999;158(1):61-70.
We have hypothesized that differences in ocular irritancy are related to differences in extent of initial injury and that, regardless of the processes leading to tissue damage, extent of injury is the primary factor that determines the final outcome of ocular irritation. In previous in vivo confocal microscopic (CM) studies we identified quantifiable differences in the extent of corneal injury occurring with four surfactants (three anionic, one cationic) known to cause different levels of ocular irritation and demonstrated that extent of initial corneal injury was related to the magnitude of cell death. The purpose of this study was to assess the applicability of this hypothesis to a broad sampling of surfactants. Specifically, initial corneal changes induced by seven different surfactants (one anionic, three cationic, three nonionic) were measured by in vivo CM and cell death was measured by an ex vivo live/dead assay. The right eye of each rabbit was treated by placing 10 microl of a surfactant directly on the cornea. Eyes were examined macroscopically and scored for irritation at 3 h and 1 day. At 3 h and 1 day, in vivo CM was used to examine the corneas and quantitate epithelial cell size, epithelial thickness, corneal thickness, and depth of stromal injury. At 3 h and/or at 1 day, corneas were removed and excised regions were placed in culture media containing 2 microM calcein AM and 4 microM ethidium homodimer. Using laser scanning CM, the number of dead epithelial and/or stromal cells in a 300 x 300 x 170-microm3 (xyz) volume of the cornea was determined. In vivo CM and live/dead assay findings revealed three surfactants to affect only the epithelium, three surfactants to affect the epithelium and superficial stroma, and one surfactant to affect the epithelium and deep stroma. Extent of initial corneal injury reflected level of ocular irritation, and magnitude of cell death was related to the extent of initial corneal injury. These findings are consistent with those for known slight, mild, and moderate to severe irritants, respectively. They suggest that our hypothesis is broadly applicable to surfactants. Additionally, we believe these surfactants should be included as part of a new "gold standard" for use in developing and validating in vitro tests to replace the use of animals in ocular irritancy testing. Copyright 1999 Academic Press.

Niimi T, Seimiya M, Kloter U, Flister S, Gehring WJ. **Direct regulatory interaction of the eyeless protein with an eye-specific enhancer in the sine oculis gene during eye induction in Drosophila.** Development 1999;126(10):2253-60.
The Pax-6 gene encodes a transcription factor with two DNA-binding domains, a paired and a homeodomain, and is expressed during eye morphogenesis and development of the nervous system. Pax-6 homologs have been isolated from a wide variety of organisms ranging from flatworms to humans. Since loss-of-function mutants in insects and mammals lead to an eyeless phenotype and Pax-6 orthologs from distantly related species are capable of inducing ectopic eyes in Drosophila, we have proposed that Pax-6 is a universal master control gene for eye morphogenesis. To determine the extent of evolutionary conservation of the eye morphogenetic pathway, we have begun to identify subordinate target genes of Pax-6. Previously we have shown that expression of two genes, sine oculis (so) and eyes absent (eya), is induced by eyeless (ey), the Pax-6 homolog of Drosophila. Here we present evidence from ectopic expression studies in transgenic flies, from transcription activation studies in yeast, and from gel shift assays in vitro that the EY protein activates transcription of sine oculis by direct interaction with an eye-specific enhancer in the long intron of the so gene.

Okamoto Y, Ohkoshi K, Itagaki H, Tsuda T, Kakishima H, Ogawa T, Kasai Y, Ohuchi J, Kojima H, Kurishita A, et al. **Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (3) Evaluation of the haemolysis test.** Toxicol In Vitro 1999;13(1):115-24.
BIOSIS COPYRIGHT: BIOL ABS. The haemolysis test using sheep red blood cells (RBC) was evaluated as an alternative method to the Draize rabbit eye irritation test (Draize test) by six to nine laboratories. The participating laboratories performed the test according to the standard operating procedure (SOP). Thirty-eight cosmetic ingredients and isotonic sodium chloride solution were used as test substances in this validation study. The concentrations of the test substances that induced 50% haemolysis (HC50 value) was obtain average values of coefficient of variation (CV) was 37%. The correlation coefficient and Spearman's rank correlation between the HC50 value and maximum average Draize total score (MAS) were -0.631 and 0.641, respectively. The equivalence ratio between the haemolysis test and MAS was 70.0% when MAS 15 was set as the in vivo cut-off point. On the other hand, strong irritants (MAS : 50) could be correctly classified by this method. These results suggest that the haemolysis test might be applied to.

Okumura H, Arashima M, Ohuchi J, Kasai Y, Tsukumo K, Kakishima H, Kotani M, Kojima H, Kurishita A, Hayashi M, et al. **Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (10) Evaluation of cytotoxicity test on CHL cells.** Toxicol In Vitro 1999;13 (1):199-208.
BIOSIS COPYRIGHT: BIOL ABS. The present interlaboratory validation study was performed in order to evaluate the use of Chinese hamster lung cell lines that employs crystal violet staining (CHL-CVS) as an alternative cytotoxicity test to the Draize eye irritation test (Draize test) for cosmetic ingredients. Ten substances, nine of which were surfactants, were evaluated at seven laboratories in the first phase of the validation study; 15 substances including dyes and lipids were evaluated at seven laboratories in the second (CV) for EC50s was 35.6%, which was considered to be within a tolerable range. The correlation coefficient and the Spearman's rank correlation coefficient between the in vitro and in vivo tests were -0.729 and 0.709, respectively. The prediction ability of the proposed method was assessed from the linear regression line for a MAS cut-off point of 15. According to this analysis, four substances (two alcohols and two acids) were determined to be false negative. The present study revealed the follo HL-CVS might have a potential to predict the Draize MAS if definite criteria can be established for the compounds to be applicable.

Pape W J, Pfannenbecker U, Argembeaux H, Bracher M, Esdaile DJ, Hagino S, Kasai Y, Lewis RW. **COLIPA validation project on in vitro eye irritation tests for cosmetic ingredients and finished products (Phase I): the red blood cell test for the estimation of acute eye irritation potentials. Present status.** Toxicol In Vitro 1999;13(2):343-54.
BIOSIS COPYRIGHT: BIOL ABS. The red blood cell test (RBC test) is part of the COLIPA Validation Project on Alternatives to Draize Eye Irritation. It shows good intra- and interlaboratory reproducibility (reliability) and represents one of the promising in vitro alternatives of this project with a good fit to prediction models (relevance) for the assessment of acute ocular irritancy caused by certain classes of chemicals (mainly surfactants) and formulations. Results obtained during the period of test development, prevalida metric changes in the haemoglobin absorption at 541 nm. The protocol also includes a set of prediction models (PM). One PM is designed to predict three classes of irritancy (classification model)

based on both end-points and the three other PMs are designed to predict modified maximum average scores (MMAS) by algorithms based on data from cellular lysis only. These three PMs (with prediction intervals (PIs)) are: (i) for surfactant ingredients, (ii) for surfactant containing finished products, a the estimation of acute eye irritation potential of surfactant-containing finished products.

Schmut O, Faulborn J, Trummer G. **[Quantifying the damage to conjunctival and corneal cell cultures caused by uv light using CASY (Cell Analysis System). A method for reducing animal experiments]**. Ophthalmologe 1999;96(6):375-81. (Ger)
BACKGROUND: By depletion of stratospheric ozone, enhanced levels of UV radiation reach the surface of the earth. Exposure of the anterior parts of the eye to UV radiation leads to irritation of the conjunctival and corneal cells. METHOD: By the CASY (cell analysis) system the influence of UV radiation on cultures of conjunctival and corneal cells was observed by determination of the cell counts, cell diameter, and the cell volume. RESULTS: By comparing these parameters with the control, damage of the conjunctival and corneal cells by UV radiation can be determined within 2 s of exposure of the cells in quartz glass vials to the UV light. CONCLUSION: By the CASY system the dramatic influence of UV radiation on cells of the anterior parts of the eye can be determined. This system enables objective statements on the cytotoxicity of radiation, chemical substances and drugs on cell cultures without the use of radioactive methods, complicated determination of cell metabolism or staining methods which are difficult to standardize. Also, studies on animals can be reduced by the CASY system.

Southee JA, Mcpherson JP, Osborne R, Carr GJ, Rasmussen E. **The performance of the tissue equivalent assay using the Skin2TM ZK1200 model in COLIPA International Validation Study on Alternatives to the Draize Eye Irritation Test.** Toxicol In Vitro 1999;13(2):355-73.
BIOSIS COPYRIGHT: BIOL ABS. The tissue equivalent assay (TEA) (Osborne et al., 1995) was used to evaluate 55 mixed ingredients and formulations in the COLIPA International Validation Study on Alternatives to the Draize Rabbit Eye Irritation Test (Brantom et al., 1997). The TEA can be used to test all types of materials since it uses a topical application approach and is not limited to only testing liquid or soluble materials. A prediction model (PM) for the test was developed using historical eye irritation data from a tot t of coded materials and the results of both laboratories were compared to the initial PM. The TEAmet the reliability criteria of the validation study in reproducing the predefined PM in both laboratories, and a good relationship between predicted and observed Draize MMAS values was obtained (r = 0.906 and r = 0.850). Good correlations were maintained when separate analyses were made of the formulations and ingredients included in the test set. Good relationships between the in vitro endpoint an s suggested a sensitivity of the model to subtle differences in application techniques, and in handling and timing. Taken together, these results indicate the utility of the TEA test for these types of substances and the need to more fully address the issue of interlaboratory reproducibility.

Tani N, Kinoshita S, Okamoto Y, Kotani M, Itagaki H, Murakami N, Sugiura S, Usami M, Kato K, Kojima H, et al. **Interlaboratory validation of the in vitro eye irritation tests for cosmetic ingredients. (8) Evaluation of cytotoxicity tests on SIRC cells.** Toxicol In Vitro 1999;13(1):175-87.
BIOSIS COPYRIGHT: BIOL ABS. Two common assays, the neutral red uptake assay (SIRC-NRU) and the crystal violet staining assay (SIRC-CVS), were evaluated as alternatives to the Draize eye irritation test (Draize test).The cytotoxicity of thirty-eight cosmetic ingredients as well as a physiological saline solution was determined on SIRC cells at five to seven laboratories. SIRC-NRU and SIRC-CVS were

performed according to the common standard operating procedure (SOP). The 50% effective concentration (EC50) was determined for r = -0.816 (n = 30), SIRC-CVS: r = -0.805 (n = 29)). Both methods could be applied to water-insoluble substances and dyes. However, strong acids, alkanolamines and alcohols had a tendency to deviate from the linear regression lines which were obtained from the in vivo and in vitro data for both methods in the present study. These results suggest that cytotoxicological testing on SIRC cells may provide an alternative method to the Draize test for cosmetic ingredients.

Zanvit A, Meunier PA, Clothier R, Ward R, Buiatti-Tcheng M. **Ocular irritancy assessment of cosmetics formulations and ingredients: fluorescein leakage test.** Toxicol In Vitro 1999;13(2):385-91.
BIOSIS COPYRIGHT: BIOL ABS. During a COLIPA multicentre study carried out on 'Alternatives to Eye Irritation' two laboratories undertook the evaluation of the samples with the fluorescein leakage test (FLT). The lead laboratory (L'Oreal) proposed a prediction model (PM), which converts the in vitro data from this assay to a prediction of eye irritation. All the surfactant-containing substances were tested using the FLT if they were soluble in Hanks' balanced salt solution (HBSS). Briefly, confluent MDCK cells were exposed the in vivo classification (linear Kappa: 0.87 : 0.17 and 0.75 : 0.25). Where there were misclassifications, the category assigned was only one different from the observed in vivo score. There was generally good agreement between the two laboratories in this study. The model seems to be more appropriate for evaluation of non-irritants or moderate irritants than for severe irritants. This is relevant for the selection of cosmetic ingredients. Overall, the results of the FLT assay appeared to be e.

PHARMACOKINETIC AND MECHANISTIC STUDIES

Abe K, Saito H. **Both oxidative stress-dependent and independent effects of amyloid beta protein are detected by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay.** Brain Res 1999;830(1):146-54.
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay has been widely used for evaluating amyloid beta protein (Abeta) toxicity. However, the potency of Abeta in inhibiting cellular MTT reduction and the underlying mechanism have been reported with some discrepancies among researchers. To understand what makes such discrepancies, the effect of Abeta detected by MTT reduction assay was re-examined in detail by using cultured rat hippocampal neurons. Micromolar concentrations (>10 microM) of Abeta caused a decrease in cell viability, which resulted in a decrease in MTT reduction per well regardless of assay time. The micromolar Abeta-induced decrease of cellular MTT reduction was significantly attenuated by antioxidants (catalase, propyl gallate or Trolox). On the other hand, nanomolar Abeta did not affect cellular MTT reduction activity at an initial stage of assay (<1 h), and decreased the total production of MTT formazan by accelerating the exocytosis of MTT formazan when MTT assay was performed for a longer time (>2 h). The assay time-dependent, nanomolar Abeta-induced decrease of cellular MTT reduction was not at all affected by antioxidants. Furthermore, subtoxic concentration of H2O2 failed to mimic the effect of nanomolar Abeta on MTT reduction. These results indicate that micromolar Abeta-induced, oxidative cell death is detected by MTT assay regardless of assay time, whereas nanomolar Abeta-induced acceleration of MTT formazan exocytosis is not mediated by oxidative stress and detected only when MTT assay is performed for a

longer time. The time of MTT assay should be properly chosen depending on the purpose of the study. Copyright 1999 Elsevier Science B.V.

Abernathy CO, Liu YP, Longfellow D, Aposhian HV, Beck B, Fowler B, Goyer R, Menzer R, Rossman T, Thompson C, et al. **Arsenic: health effects, mechanisms of actions, and research issues.** Environ Health Perspect 1999;107(7):593-7.
A meeting on the health effects of arsenic (As), its modes of action, and areas in need of future research was held in Hunt Valley, Maryland, on 22-24 September 1997. Exposure to As in drinking water has been associated with the development of skin and internal cancers and noncarcinogenic effects such as diabetes, peripheral neuropathy, and cardiovascular diseases. There is little data on specific mechanism (s) of action for As, but a great deal of information on possible modes of action. Although arsenite [As (III)] can inhibit more than 200 enzymes, events underlying the induction of the noncarcinogenic effects of As are not understood. With respect to carcinogenicity, As can affect DNA repair, methylation of DNA, and increase radical formation and activation of the protooncogene c-myc, but none of these potential pathways have widespread acceptance as the principal etiologic event. In addition, there are no accepted models for the study of As-induced carcinogenesis. At the final meeting session we considered research needs. Among the most important areas cited were a) As metabolism and its interaction with cellular constituents; b) possible bioaccumulation of As; c) interactions with other metals; d) effects of As on genetic material; e) development of animal models and cell systems to study effects of As; and f) a better characterization of human exposures as related to health risks. Some of the barriers to the advancement of As research included an apparent lack of interest in the United States on As research; lack of relevant animal models; difficulty with adoption of uniform methodologies; lack of accepted biomarkers; and the need for a central storage repository for stored specimens.

Akao Y, Nakagawa Y, Akiyama K. **Arsenic trioxide induces apoptosis in neuroblastoma cell lines through the activation of caspase 3 in vitro.** FEBS Lett 1999;455(1-2):59-62.
Arsenic trioxide (As2O3) induces clinical remission in acute promyelocytic leukemia, even in all-trans retinoic acid-refractory cases, with minimal toxicity at low (1-2 microM) concentration. We exposed various neuroblastoma cell lines to As2O3 at a concentration of 2 microM: as a result, seven of 10 neuroblastoma cell lines underwent apoptosis characterized by morphological changes and nucleosomal DNA fragmentation. As2O3-induced apoptosis in neuroblastoma cells was shown to occur through the activation of caspase 3, as judged from Western blot analysis and apoptosis inhibition assay. It seemed that the sensitivity of neuroblastoma cells to As2O3 was inversely proportional to their intracellular level of reduced glutathione. Taken together these results indicate that As2O3 would be a candidate as a therapeutic agent for treatment of neuroblastoma, which is a solid tumor, not only by systemic therapy but also by local therapy.

Andersen ME, Gearhart JM, Clewell HJ 3rd. **Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese.** Neurotoxicol 1999;20(2-3):161-71.
Manganese (Mn)-deficiency or Mn-excess can lead to adverse biological consequences. Central nervous system tissues, rich in dopaminergic neurons, are the targets whether the Mn gains entrance by inhalation, oral ingestion, or intravenous administration. Risk assessments with Mn need to ensure that brain concentrations in the globus pallidus and striatum stay within the range of normal. This paper first provides a critical review of the biological factors that determine the disposition of Mn in tissues within

the body. Secondly, it outlines specific data needs for developing a physiologically based pharmacokinetic (PBPK) model for Mn to assist in conducting risk assessments for inhaled and ingested Mn. Uptake of dietary Mn appears to be controlled by several dose-dependent processes: biliary excretion, intestinal absorption, and intestinal elimination. Mn absorbed in the divalent form from the gut via the portal blood is complexed with plasma proteins that are efficiently removed by the liver. Absorption of Mn via inhalation, intratracheal instillation or intravenous infusions bypasses the control processes in the gastrointestinal tract. After absorption into the blood system by these alternate routes, Mn is apparently oxidized by ceruloplasmin and the trivalent Mn binds to the iron carrying protein, transferrin. Brain uptake of Mn occurs via transferrin receptors located in various brain regions. Transferrin-bound trivalent Mn is not as readily removed by the liver, as are protein complexes with divalent Mn. Thus, Mn delivered by these other dose routes would be available for uptake into tissues for a longer period of time than the orally administered Mn, leading to quantitative differences in tissue uptake for different dose routes. Several important data gaps impede organizing these various physiological factors into a multi-dose route PK model for Mn. They include knowledge of (1) oxidation rates of Mn in blood, (2) uptake rates of protein-bound forms of Mn by the liver, (3) neuronal transfer rates within the CNS, and (4) quantitative analyses of the control processes that regulate uptake of ingested Mn by the intestines and liver. These data gaps are the main obstacles to developing a risk assessment strategy for Mn that considers contributions of both inhalation and ingestion of this essential nutrient in determining brain Mn concentrations.

Barton HA, Bull R, Schultz I, Andersen ME. **Dichloroacetate (DCA) dosimetry: interpreting DCA-induced liver cancer dose response and the potential for DCA to contribute to trichloroethylene-induced liver cancer.** Toxicol Lett 1999;106(1):9-21.
Pharmacokinetic studies with dichloroacetate (DCA) provide insights into the likelihood that trichloroethylene-induced liver cancers arise from formation of DCA as a metabolite and the mode of action by which DCA induces liver cancer. A simple physiologically based pharmacokinetic model was developed to analyze DCA blood concentration data from mice unexposed to or pre-treated with DCA. The large first pass metabolism of DCA in the liver is significantly reduced by DCA pretreatment. Because DCA inhibits its own metabolism, large increases in area under the blood concentration curve occur at lower doses than would be predicted from single-dose pharmacokinetic studies with naive mice. The dose metrics associated with the incidence of liver tumors in contrast to the multiplicity of tumors per animal may be different, suggesting potentially different roles in the cancer process for DCA versus its metabolites. By linking a model for trichloroethylene (TCE) pharmacokinetics with the DCA model, maximum levels of DCA potentially produced from TCE were estimated to be at or below the analytical chemistry detection limits. In addition, the predicted levels of DCA would be too small to produce the observed liver cancers following corn oil gavage exposure of mice to TCE.

Baskin SI, Porter DW, Rockwood GA, Romano JA Jr, Patel HC, Kiser RC, Cook CM, Ternay AL Jr. **In vitro and in vivo comparison of sulfur donors as antidotes to acute cyanide intoxication.** J Appl Toxicol 1999;19(3):173-83.
Antidotes for cyanide (CN) intoxication include the use of sulfane sulfur donors (SSDs), such as thiosulfate, which increase the conversion of CN to thiocyanate by the enzyme rhodanese. To develop pretreatments that might be useful against CN, SSDs with greater lipophilicity than thiosulfate were

synthesized and assessed. The ability of SSDs to protect mice against 2LD50 of sodium cyanide (NaCN) administered either 15 or 60 min following administration of an SSD was assessed. To study the mechanism of action of the SSD, the candidate compounds were examined in vitro for their effect on rhodanese and 3-mercaptopyruvate sulfurtransferase (MST) activity under increasing SSD concentrations. Tests were conducted on nine candidate SSDs: ICD1021 (3-hydroxypyridin-2-yl N-[(N-methyl-3-aminopropyl)]-2-aminoethyl disulfide dihydrochloride), ICD1022, (3-hydroxypyridin-2-yl N-[(N-methyl-3-aminopropyl)]-2-aminoethyl disulfide trihydrochloride), ICD1584 (diethyl tetrasulfide), ICD1585 (diallyl tetrasulfide), ICD1587 (diisopropyl tetrasulfide); ICD1738 (N-(3-aminopropyl)-2-aminoethyl 2-oxopropyl disulfide dihydrochloride), ICD1816 (3,3'-tetrathiobis-N-acctyl-L-alanine), ICD2214 (2-aminoethyl 4-methoxyphenyl disulfide hydrochloride) and ICD2467 (bis(4-methoxyphenyl) disulfide). These tests demonstrated that altering the chemical substituent of the longer chain sulfide modified the ability of the candidate SSD to protect against CN toxicity. At least two of the SSDs at selected doses provided 100% protection against 2LD50 of NaCN, normally an LD99. All compounds were evaluated using locomotor activity as a measure of potential adverse behavioral effects. Positive hypoactivity relationships were found with several disulfides but none was found with ICD1584, a tetrasulfide. Separate studies suggest that the chemical reaction of potassium cyanide (KCN) and cystine forms the toxic metabolite 2-iminothiazolidine-4-carboxylic acid. An alternative detoxification pathway, one not primarily involving the sulfur transferases. may be important in pretreatment for CN intoxication. Although studies to elucidate the precise mechanisms are needed. it is clear that these newly synthesized compounds provide a new rationale for anti-CN drugs, with fewer side-effects than the methemoglobin formers.

Bastos V L, Filho M V, Rossini A, Bastos JC. **The activation of parathion by brain and liver of a Brazilian suckermouth benthic fish shows comparable in vitro kinetics.** Pestic Biochem Physiol 1999;64(3):149-56.
BIOSIS COPYRIGHT: BIOL ABS. A constant influx of parathion into livers of benthic fishes could overflow their ability to both activate (into paraoxon) and detoxify (through hydrolysis) molecules and metabolites of this phosphorothioate. This could allow parathion to be activated in brain cells with consequent nervous intoxication. Assay of acetylcholinesterase (AChE) enzyme activities allowed the nanomolar quantification of paraoxon produced from parathion in vitro by liver and brain homogenates of cascudo, a Brazilian suc hat of the liver tissues, when parathion concentrations are lower than 30 muM. Moreover, cascudo brain would be faster than liver to promote oxidative desulfuration of parathion between 35 and 55 muM. As no parathion cleavage was measured using brain or liver homogenates of cascudo it became clear that direct hydrolysis of parathion would not work as a detoxification process in these tissues. These results may reveal typical processes of organophosphate metabolism present in benthic fish which c.

Blasiak J, Gloc-Fudala E, Trzeciak A. **Formation of DNA crosslinks in human lymphocytes by acetaldehyde revealed by the comet assay.** Cell Mol Biol Lett 1999;4(2):181-7.
BIOSIS COPYRIGHT: BIOL ABS. Ethyl alcohol can be mutagenic, cancerogenic and teratogenic in man and its mutagenicity can be attributed to its first and major metabolite, acetaldehyde, which was reported to form adducts with DNA and proteins and DNA strand breaks. It was suggested that DNA crosslinks can be predominant DNA adducts of acetaldehyde but these results were obtained with the

alkaline elution technique which provides no information on the extent of DNA damage at individual cell level. The comet assay is a techniq yde-treated cells was observed. Similar results were obtained when a recognized DNA crosslinking agent, formaldehyde was used. The results obtained suggest that acetaldehyde may form crosslinks with DNA in human lymphocytes. The nature of the crosslinks remains unknown and needs further investigations.

Bogdanffy MS, Sarangapani R, Plowchalk DR, Jarabek A, Andersen ME. **A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity.** Toxicol Sci 1999;51 (1):19-35.
The 1990 Clean Air Act Amendments require that health risk from exposure to vinyl acetate be assessed. Vinyl acetate is a nasal carcinogen in rats, but not mice, and induces olfactory degeneration in both species. A biologically based approach to extrapolating risks of inhalation exposure from rats to humans was developed, which incorporates critical determinants of interspecies dosimetry. A physiologically based pharmacokinetic (PBPK) model describing uptake and metabolism of vinyl acetate in rat nose was validated against nasal deposition data collected at three airflow rates. The model was also validated against observations of metabolically derived acetaldehyde. Modifying the rat nose model to reflect human anatomy created a PBPK model of the human nose. Metabolic constants from both rats and humans specific for vinyl acetate and acetaldehyde metabolism enabled predictions of various olfactory tissue dosimeters related to the mode of action. Model predictions of these dosimeters in rats corresponded well with observations of vinyl acetate toxicity. Intracellular pH (pHi) of olfactory epithelial cells was predicted to drop significantly at airborne exposure concentrations above the NOAEL of 50 ppm. Benchmark dose methods were used to estimate the ED10 and LED10 for olfactory degeneration, the precursor lesion thought to drive cellular proliferation and eventually tumor development at excess cellular acetaldehyde levels. A concentration x time adjustment was applied to the benchmark dose values. Human-equivalent concentrations were calculated by using the human PBPK model to predict concentrations that yield similar cellular levels of acetic acid, acetaldehyde, and pHi. After the application of appropriate uncertainty factors, an ambient air value of 0.4 to 1.0 ppm was derived. The biologically based approach supports a workplace standard of 10 ppm.

Bonnabry P, Sievering J, Leemann T, Dayer P. **Quantitative drug interactions prediction system (Q-DIPS): a computer-based prediction and management support system for drug metabolism interactions.** Eur J Clin Pharmacol 1999;55(5):341-7.
OBJECTIVE: Drug biotransformation and interactions are a major source of variability in the response to drugs. The superfamily of cytochromes P450 plays a key role in this phenomenon but, because of the complexity of interactions between drugs and isozymes, it becomes more and more difficult for clinicians to master the knowledge required to predict the occurrence of such drug interactions. To predict and help manage the occurrence of cytochrome P450-dependent interactions, we developed an original computer application: Q-DIPS (quantitative drug interactions prediction system). METHODS: A multidisciplinary work team was created, associating clinical pharmacologists, pharmacists and a computer scientist. Major steps of investigation were: (1) the creation of a database to collect qualitative and quantitative data describing substrates, inhibitors and inducers of specific cytochrome P450 isozymes, with quality assessments; (2) the development of multi-access to these data and (3) their incorporation into extrapolation systems allowing the prediction of in vivo drug interactions on the basis

of in vitro data. As an example, prediction and validation studies of CYP3A4 inhibition by ketoconazole and fluconazole will be discussed. RESULTS: Q-DIPS gives up-to-date information, in dynamic tables, describing which specific P450 isozymes metabolise a given drug, as well as which drugs may inhibit or induce a given isozyme. To better answer common clinical questions and help to rapidly evaluate the risk of interactions, it is possible to obtain an overview of substances causing interactions with a specific drug or to focus on drugs taken by a patient ("clinical case"). For each question, key references, relevant quantitative data and quality indices are easily accessible. Two modules allowing input with commercial names and the anatomical therapeutic chemical classification were also included. On the basis of enzymatic and pharmacokinetic data generated in vitro or collected in vivo, the extrapolation module integrates quantitative models to predict the impact of a treatment on enzymatic activities. The simplest model predicted a strong but fluctuating inhibition of CYP3A4 by ketoconazole, whereas the impact of fluconazole was lower. Validations with published in vivo data suggested an appropriate prediction of the risk. CONCLUSION: The current Q-DIPS prototype shows promising potential for helping to improve the management of drug interactions involving metabolism. Validation of extrapolation techniques need to be completed, in view of including important factors such as intrahepatocyte drug accumulation, contribution of metabolites to inhibition as well as in vitro non-specific binding to microsomal proteins. The final goal will be to help select the most judicious clinical studies to be performed so as to avoid useless, expensive and unethical investigations in man.

Carmel Z, Amsallem H, Metioui M, Dehaye JP, Moran A. **Are salivary glands cell lines in culture a good model for purinergic receptors in salivary glands?** Arch Oral Biol 1999;44(Suppl 1):63-6.
A major obstacle in studying the physiological and biochemical processes of salivary secretion is the lack of a good ductal cell line model. HSY, an immortalised cell line originating from human parotid gland intercalated ducts, provides a possible model for purinergic mechanisms in ductal cells. Unlike the biphasic dose response to ATP of isolated submandibular ductal cells, the rise in [Ca2+]i in HSY cells shows single Michaelis-Menten kinetics with an apparent Ka of 0.8 microM. Pre-incubation with thapsigargin inhibited the ATP induced [Ca2+]i rise. Both ATP (10 microM) and carbachol (100 microM) increased IP3 production. Intercalated duct cells may differentiate into acinar or ductal cells in response to appropriate stimuli from extracellular matrix We therefore attempted to induce a duct-like phenotype in the striated duct-derived HSY cells by growing them on microcarrier beads coated with type I collagen. In Ca-containing medium cells grown on all substrates showed similar responses to ATP. In contrast, in Ca-free medium, [Ca2+]i rose only slightly in cells grown on beads relative to those on glass. This probably resulted from reduced IP3 production. Carbachol also induced a much smaller increase in [Ca2+]i and less IP3 production in cells grown on Cytodex-3. The HSY response to purinergic stimuli by an increase in [Ca2+]i and IP3 means that they can be used to study the metabotropic purinergic pathway. The impairment in the HSY responses grown on Cytodex-3 can be used to probe phosposinositol signal transduction in salivary cells.

Carpenter RL. **Aerosol deposition modeling using ACSL.** Drug Chem Toxicol 1999;22(1):73-90.
An aerosol deposition model has been written for inclusion into physiologically based pharmacokinetic (PBPK) models, allowing PBPK model based risk assessments to be performed for aerosolized materials. Previously, PBPK models could only treat inhaled gases and vapors. The deposition model employs a semi-empirical equation to describe extrathoracic deposition and employs data concerning the

geometry of the thoracic conducting airways as well as that of the gas exchange regions of the lung to compute the deposited aerosol mass based on aerosol diffusion, sedimentation, and impaction. Provisions are made to allow calculations for polydisperse aerosols whose size distribution and mass vary with time. Variations in the model subject's respiration can be accommodated through selection of respiratory parameters at model startup as well as through consideration of carbon dioxide stimulation of respiration. The model is compared with other similar calculations and experimental data to validate the calculations. An example model application is presented in the form of a comparison of two inhalation atmospheres, one from an inhalation toxicity study and one from a similar atmosphere produced for fire extinguishing agent testing.

Chen Y, Schindler M, Simon SM. **A mechanism for tamoxifen-mediated inhibition of acidification.** J Biol Chem 1999;274(26):18364-73.
Tamoxifen has been reported to inhibit acidification of cytoplasmic organelles in mammalian cells. Here, the mechanism of this inhibition is investigated using in vitro assays on isolated organelles and liposomes. Tamoxifen inhibited ATP-dependent acidification in organelles from a variety of sources, including isolated microsomes from mammalian cells, vacuoles from Saccharomyces cerevisiae, and inverted membrane vesicles from Escherichia coli. Tamoxifen increased the ATPase activity of the vacuolar proton ATPase but decreased the membrane potential (Vm) generated by this proton pump, suggesting that tamoxifen may act by increasing proton permeability. In liposomes, tamoxifen increased the rate of pH dissipation. Studies comparing the effect of tamoxifen on pH gradients using different salt conditions and with other known ionophores suggest that tamoxifen affects transmembrane pH through two independent mechanisms. First, as a lipophilic weak base, it partitions into acidic vesicles, resulting in rapid neutralization. Second, it mediates coupled, electroneutral transport of proton or hydroxide with chloride. An understanding of the biochemical mechanism(s) for the effects of tamoxifen that are independent of the estrogen receptor could contribute to predicting side effects of tamoxifen and in designing screens to select for estrogen-receptor antagonists without these side effects.

Chusacultanachai S, Glenn KA, Rodriguez AO, Read EK, Gardner JF, Katzenellenbogen BS, Shapiro DJ. **Analysis of estrogen response element binding by genetically selected steroid receptor DNA binding domain mutants exhibiting altered specificity and enhanced affinity.** J Biol Chem 1999;274 (33):23591-8.
To analyze the role of amino acids in the steroid receptor DNA binding domain (DBD) recognition helix in binding of the receptor to the estrogen response element (ERE), we adapted the powerful P22 challenge phage selection system for use with a vertebrate protein. We used the progesterone receptor DNA binding domain and selected for mutants that gained the ability to bind to the ERE. We used a mutagenesis protocol based on degenerate oligonucleotides to create a large and diverse pool of mutants in which 10 nonconsensus amino acids in the DNA recognition helix of the progesterone receptor DNA binding domain were randomly mutated. After a single cycle of modified P22 challenge phage selection, 37 mutant proteins were identified, all of which lost the ability to bind to the progesterone response element. In gel mobility shift assays, approximately 70% of the genetically selected mutants bound to the consensus ERE with a >4-fold higher affinity than the naturally occurring estrogen receptor DBD. In the P-box region of the DNA recognition helix, the selected mutants contained the amino acids found in the wild-type estrogen receptor DBD, as well as other amino acid combinations seen in naturally

occurring steroid/nuclear receptors that bind the aGGTCA half-site. We also obtained high affinity DBDs with Trp(585) as the first amino acid of the P-box, although this is not found in the known steroid/nuclear receptors. In the linker region between the two zinc fingers, G597R was by far the most common mutation. In transient transfections in mammalian cells using promoter interference assays, the mutants displayed enhanced affinity for the ERE. When linked to an activation domain, the transfected mutants activated transcription from ERE-containing reporter genes. We conclude that the P-box amino acids can display considerable variation and that the little studied linker amino acids play an important role in determining affinity for the ERE. This work also demonstrates that the P22 challenge phage genetic selection system, modified for use with a mammalian protein, provides a novel, single cycle selection for steroid/nuclear receptor DBDs with altered specificity and greatly enhanced affinity for their response elements.

Clement B, Boucher JL, Mansuy D, Harsdorf A. **Microsomal formation of nitric oxide and cyanamides from non-physiological N-hydroxyguanidines: N-hydroxydebrisoquine as a model substrate.** Biochem Pharmacol 1999;58(3):439-45.
The microsomal oxidative transformation of a non-physiological N-hydroxyguanidine was demonstrated for the first time for N-hydroxydebrisoquine as a model substrate (Clement et al., Biochem Pharmacol 46: 2249-2267, 1993). The objective of the present work was to further compare this reaction with the analogous oxidation of arginine via N-hydroxyarginine to citrulline and nitric oxide. The oxidation of N-hydroxydebrisoquine by liver microsomes from rats pretreated with dexamethasone not only produced nitric oxide and the urea, but also the cyanamide derivative as the main metabolite. The low stability of the cyanamide derivative, which easily hydrolyzed to the urea derivative, was noted. The formation of all compounds required cosubstrate and the enzyme source. Experiments with catalase, superoxide dismutase, and H2O2 showed that the O2- formed from the enzyme and the substrate apparently participated in the reaction. While the N-hydroxylation of the guanidine involves the usual monooxygenase activity of cytochrome P-450 (Clement et al., Biochem Pharmacol 46: 2249-2267, 1993), the resultant N-hydroxyguanidine decoupled the monooxygenase. Nitric oxide was detected by the oxyhemoglobin assay. To examine the influence of enzymatically formed nitric oxide on the formation of the metabolites, the N-hydroxydebrisoquine was incubated with SIN-1 as nitric oxide donor under aerobic conditions. It was again possible to detect the cyanamide and urea derivatives, with the latter as main metabolite. It was concluded that the microsomal transformation of N-hydroxydebrisoquine produces a cyanamide and nitric oxide which reacts with N-hydroxydebrisoquine to form the urea derivative. The purely chemical reaction of the unsubstituted N-hydroxyguanidine with nitric oxide gave similar results (Fukuto et al., Biochem Pharmacol 43: 607-613, 1992). In conclusion, similarities (formation of a urea derivative) and differences (formation of a cyanamide derivative) between the physiological oxidation of N-hydroxy-L-arginine by nitric oxide synthases and non-physiological N-hydroxyguanidines by cytochrome P-450 were observed. Furthermore, non-physiological N-hydroxyguanidines can be regarded as nitric oxide donors.

Collins AS, Sumner SC, Borghoff SJ, Medinsky MA. **A physiological model for tert-amyl methyl ether and tert-amyl alcohol: hypothesis testing of model structures.** Toxicol Sci 1999;49(1):15-28.
The oxygenate tert-amyl methyl ether (TAME) is a gasoline fuel additive used to reduce carbon monoxide in automobile emissions. To evaluate the relative health risk of TAME as a gasoline additive,

information is needed on its pharmacokinetics and toxicity. The objective of this study was to use a physiologically-based pharmacokinetic (PBPK) model to describe the disposition of TAME and its major metabolite, tert-amyl alcohol (TAA), in male Fischer-344 rats. The model compartments for TAME and TAA were flow-limited. The TAME physiological model had 6 compartments: lung, liver, rapidly perfused tissues, slowly perfused tissues, fat, and kidney. The TAA model had 3 compartments: lung, liver, and total-body water. The 2 models were linked through metabolism of TAME to TAA in the liver. Model simulations were compared with data on blood concentrations of TAME and TAA taken from male Fischer-344 rats during and after a 6-hour inhalation exposure to 2500, 500, or 100 ppm TAME. The PBPK model predicted TAME pharmacokinetics when 2 saturable pathways for TAME oxidation were included. The TAA model, which included pathways for oxidation and glucuronide conjugation of TAA, underpredicted the experimental data collected at later times postexposure. To account for biological processes occurring during this time, three hypotheses were developed: nonspecific binding of TAA, diffusion-limited transport of TAA, and enterohepatic circulation of TAA glucuronide. These hypotheses were tested using three different model structures. Visual inspection and statistical evaluation involving maximum likelihood techniques indicated that the model incorporating nonspecific binding of TAA provided the best fit to the data. A correct model structure, based upon experimental data, statistical analyses, and biological interpretation, will allow a more accurate extrapolation to humans and, consequently, a greater understanding of human risk from exposure to TAME.

Cooke C, Hans H, Alwine JC. **Utilization of splicing elements and polyadenylation signal elements in the coupling of polyadenylation and last-intron removal.** Mol Cell Biol 1999;19(7):4971-9. Polyadenylation (PA) is the process by which the 3' ends of most mammalian mRNAs are formed. In nature, PA is highly coordinated, or coupled, with splicing. In mammalian systems, the most compelling mechanistic model for coupling arises from data supporting exon definition (2, 34, 37). We have examined the roles of individual functional components of splicing and PA signals in the coupling process by using an in vitro splicing and PA reaction with a synthetic pre-mRNA substrate containing an adenovirus splicing cassette and the simian virus 40 late PA signal. The effects of individually mutating splicing elements and PA elements in this substrate were determined. We found that mutation of the polypyrimidine tract and the 3' splice site significantly reduced PA efficiency and that mutation of the AAUAAA and the downstream elements of the PA signal decreased splicing efficiency, suggesting that these elements are the most significant for the coupling of splicing and PA. Although mutation of the upstream elements (USEs) of the PA signal dramatically decreased PA, splicing was only modestly affected, suggesting that USEs modestly affect coupling. Mutation of the 5' splice site in the presence of a viable polypyrimidine tract and the 3' splice site had no effect on PA, suggesting no effect of this element on coupling. However, our data also suggest that a site for U1 snRNP binding (e.g., a 5' splice site) within the last exon can negatively effect both PA and splicing; hence, a 5' splice site-like sequence in this position appears to be a modulator of coupling. In addition, we show that the RNA-protein complex formed to define an exon may inhibit processing if the definition of an adjacent exon fails. This finding indicates a mechanism for monitoring the appropriate definition of exons and for allowing only pre-mRNAs with successfully defined exons to be processed.

Coultrap SJ, Sun H, Tenner TE Jr, Machu TK. **Competitive antagonism of the mouse 5-**

hydroxytryptamine3 receptor by bisindolylmaleimide I, a "selective" protein kinase C inhibitor. J Pharmacol Exp Ther 1999;290(1):76-82.
We examined the effects of several protein kinase C (PKC) inhibitors on the murine 5-hydroxytryptamine3 (5-HT3) receptor to determine whether they acted directly on the receptor. The 5-HT-evoked currents in Xenopus laevis oocytes expressing the recombinant 5-HT3 receptor were measured with the two-electrode voltage-clamp technique. The PKC inhibitors bisindolylmaleimide I (BIM, GF109203x) and staurosporine, but not calphostin C or chelerythrine, decreased the 5-HT3 receptor-mediated currents when coapplied with 5-HT. BIM blocked 0.5 microM 5-HT-elicited currents with an IC50 value of 7 nM, whereas in the presence of 5 microM staurosporine, 42% inhibition of 0.5 microM 5-HT-mediated currents was observed. Increasing concentrations of BIM resulted in a rightward shift of the 5-HT concentration-response curve, without altering efficacy. A Schild plot was generated, which had a slope of -1.01, suggesting competitive antagonism. The Ki value of BIM was determined to be 29 nM. To confirm competitive antagonism, a competitive binding assay was performed on Sf21 insect cells infected with the mouse 5-HT3 receptor cDNA in a baculovirus expression vector. BIM completely displaced binding of the selective 5-HT3 receptor antagonist [3H]GR65630. BIM bound to the 5-HT3 receptor with a Ki value of 61 nM, which was slightly less potent than that of the selective 5-HT3 receptor antagonist MDL72222 (27 nM). The PKC inhibitor BIM is a potent competitive antagonist at the 5-HT3 receptor.

Cravedi JP, Lafuente A, Baradat M, Hillenweck A, Perdu-Durand E. **Biotransformation of pentachlorophenol, aniline and biphenyl in isolated rainbow trout (Oncorhynchus mykiss) hepatocytes: comparison with in vivo metabolism.** Xenobiotica 1999;29(5):499-509.
1. The biotransformation of pentachlorophenol (PCP), aniline and biphenyl in rainbow trout (Oncorhynchus mykiss) isolated liver cells was investigated to examine if fish hepatocytes represent a suitable alternative to the in vivo approach for studying the biotransformation of chemicals. Each compound was incubated at two concentrations (10 and 60 microM) for 2 h. For comparison, the metabolic profile of these xenobiotics was also studied in urine and bile of trout orally exposed to 1.8-4.0 mg/kg wet wt of each compound. 2. In vitro as in vivo, PCP glucuronide and to a lesser extent PCP sulphate were the metabolites formed by trout from PCP. 3. Aniline was mainly metabolized to acetanilide and to a lesser extent to 2-aminophenol by isolated hepatocytes, but neither hydroxylated acetanilide nor conjugates were found in vitro whereas they were present in bile and urine of trout treated with this chemical. 4. Trout hepatocytes metabolized biphenyl to hydroxylated and dihydroxylated products and the corresponding glucuronides. These results correlated well with the metabolic profile obtained from the bile of trout exposed to this pesticide. 5. It is concluded that although hepatocytes are well suited for several types of biotransformation studies, the fact that this system may in some cases produce a different metabolic pattern than in vivo should be considered when attempting to extrapolate in vitro to in vivo data.

Cunningham ML, Filtz TM, Harden TK. **Protein kinase C-promoted inhibition of Galpha(11)-stimulated phospholipase C-beta activity.** Mol Pharmacol 1999;56(2):265-71.
The effects of protein kinase C (PKC) activation on inositol lipid signaling were examined. Using the turkey erythrocyte model of receptor-regulated phosphoinositide hydrolysis, we developed a membrane reconstitution assay to study directly the effects of activation of PKC on the activities of Galpha(11),

independent of potential effects on the receptor or on PLC-beta. Membranes isolated from erythrocytes pretreated with 4beta-phorbol-12beta-myristate-13alpha-acetate (PMA) exhibited a decreased capacity for Galpha(11)-mediated activation of purified, reconstituted PLC-beta1. This inhibitory effect was dependent on both the time and concentration of PMA incubation and occurred as a decrease in the efficacy of GTPgammaS for activation of PLC-beta1, both in the presence and absence of agonist; no change in the apparent affinity for the guanine nucleotide occurred. Similar inhibitory effects were observed after treatment with the PKC activator phorbol-12,13-dibutyrate but not after treatment with an inactive phorbol ester. The inhibitory effects of PMA were prevented by coaddition of the PKC inhibitor bisindolylmaleimide. Although the effects of PKC could be localized to the membrane, no phosphorylation of Galpha(11) occurred either in vitro in the presence of purified PKC or in intact erythrocytes after PMA treatment. These results support the hypothesis that a signaling protein other than Galpha(11) is the target for PKC and that PKC-promoted phosphorylation of this protein results in a phosphorylation-dependent suppression of Galpha(11)-mediated PLC-beta1 activation.

De Salvia R, Fiore M, Aglitti T, Festa F, Ricordy R, Cozzi R. **Inhibitory action of melatonin on H2O2- and cyclophosphamide-induced DNA damage.** Mutagenesis 1999;14(1):107-12.
Melatonin, the pineal gland hormone known for its ability to modulate circadian rhythm, has recently been studied in its several functions. It is believed to inhibit cancer growth, to stimulate the immune system and to act as an antioxidant. In particular, this latter activity is ascribed to two different mechanisms: stimulation of radical detoxifying enzymes and scavenging of free radicals. We used this compound in mammalian cells in vitro to investigate its mechanism of action in modulating DNA damage. Cytogenetic and cytofluorimetric analyses were performed. We show that melatonin is able to modulate chromosome damage (chromosomal aberrations and sister chromatid exchanges) induced by cyclophosphamide. Conversely, its involvement in modulating oxidative processes, thereby reducing DNA damage, is less clear. In particular, melatonin is able to decrease H2O2-induced chromosomal aberrations but not sister chromatid exchanges and has been found to induce oxygen species in a cytofluorimetric test (DCFH assay).

Dejongh J, Forsby A, Houston JB, Beckman M, Combes R, Blaauboer BJ. **An integrated approach to the prediction of systemic toxicity using computer-based biokinetic models and biological in vitro test methods: overview of a prevalidation study based on the ECITTS project.** Toxicol In Vitro 1999;13(4-5):549-54.
BIOSIS COPYRIGHT: BIOL ABS. Chemical toxicity was estimated by integrating in vitro study results with physiologically-based biokinetic models for eight neurotoxic compounds (benzene, toluene, lindane, acrylamide, parathion/oxon, caffeine, diazepam and phenytoin). In vitro studies on general and specific neurotoxicity were performed and biotransformation and tissue-blood distribution studies were used in modelling the biokinetic behaviour of the compounds. Subsequently, neurotoxicity was estimated from the integrated in vi OELs for the most active compounds could only be established after consideration of additional in vitro results from the literature. The present study has generated encouraging results on the risk assessment of chemicals from in vitro studies and computer simulations and has identified some key directions for future research.

Diah SK, Smitherman PK, Townsend AJ, Morrow CS. **Detoxification of 1-chloro-2,4-dinitrobenzene in MCF7 breast cancer cells expressing glutathione S-transferase P1-1 and/or multidrug resistance**

protein 1. Toxicol Appl Pharmacol 1999;157(2):85-93.
We examined the roles of glutathione S-transferase (GST) P1-1 and the glutathione S-conjugate (GS-X) transporter, multidrug resistance protein 1 (MRP1), singly or in combination, in the detoxification of 1-chloro-2,4-dinitrobenzene (CDNB). Derivatives of MCF7 breast carcinoma cells expressing GST P1-1 and MRP1 alone or in combination were developed. Detoxification was measured in cells as formation of the glutathione conjugate of CDNB, S-(2,4-dinitrophenyl)-glutathione (DNP-SG), efflux of DNP-SG, and ultimately protection from CDNB cytotoxicity. MRP1 expression in the absence of GST P1-1 confers a three- to fourfold resistance to CDNB, which is associated with a >10-fold increase in the maximum rate of DNP-SG efflux. DNP-SG efflux in MRP1-expressing MCF7 cells was ATP-dependent and exhibited an apparent Km for DNP-SG of 95 microM. MRP1 expression alone, however, had no effect on DNP-SG formation. Combined expression of GST P1-1 and MRP1 increased the rates of DNP-SG formation when cells were exposed to 10 microM CDNB. Moreover, combined expression of GSTP1-1 with MRP1 moderately augmented MRP1-mediated resistance to CDNB but only during short term (10 min) exposures to CDNB where IC50 values were in the 8-10 microM range. In contrast, expression of GST P1-1 in the absence of MRP1 slightly sensitized cells to the toxicity of CDNB (10 min exposures), despite increasing rates of DNP-SG formation. The sensitization to CDNB in cells expressing GST P1-1 alone was associated with increased intracellular accumulation of DNP-SG, indicating that DNP-SG may contribute to CDNB toxicity. The potential toxicity of DNP-SG is also suggested by the finding that inhibition of DNP-SG formation by prior glutathione depletion confers resistance to CDNB cytotoxicity in MRP1-poor MCF7 cells. Altogether, our results demonstrate that glutathione conjugation and MRP1-mediated conjugate efflux can operate together to confer resistance to CDNB. The data indicate that MRP1-mediated conjugate efflux is required for cytoprotection from CDNB because its conjugate (DNP-SG), when present at high intracellular levels, may also be toxic to cells. Copyright 1999 Academic Press.

Dianov GL, Jensen BR, Kenny MK, Bohr VA. **Replication protein A stimulates proliferating cell nuclear antigen-dependent repair of abasic sites in DNA by human cell extracts.** Biochemistry 1999;38(34):11021-5.
Base excision repair (BER) pathway is the major cellular process for removal of endogenous base lesions and apurinic/apyrimidinic (AP) sites in DNA. There are two base excision repair subpathways in mammalian cells, characterized by the number of nucleotides synthesized into the excision patch. They are the "single-nucleotide" (one nucleotide incorporated) and the "long-patch" (several nucleotides incorporated) BER pathways. Proliferating cell nuclear antigen (PCNA) is known to be an essential factor in long-patch base excision repair. We have studied the role of replication protein A (RPA) in PCNA-dependent, long-patch BER of AP sites in human cell extracts. PCNA and RPA were separated from the other BER proteins by fractionation of human whole-cell extract on a phosphocellulose column. The protein fraction PC-FII (phosphocellulose fraction II), which does not contain RPA and PCNA but otherwise contains all core BER proteins required for PCNA-dependent BER (AP endonuclease, DNA polymerases delta, beta and DNA ligase, and FEN1 endonuclease), had reduced ability to repair plasmid DNA containing AP sites. Purified PCNA or RPA, when added separately, could only partially restore the PC-FII repair activity of AP sites. However, additions of both proteins together greatly stimulated AP site repair by PC-FII. These results demonstrate a role for RPA in PCNA-dependent BER of AP sites.

Draper AJ, Hammock BD. **Soluble epoxide hydrolase in rat inflammatory cells is indistinguishable from soluble epoxide hydrolase in rat liver.** Toxicol Sci 1999;50(1):30-5.
Soluble epoxide hydrolase (sEH) is a ubiquitous mammalian enzyme for which liver and kidney are reported to have the highest activity. We have shown that the soluble epoxide hydrolase (sEH) activity present in rat neutrophils and macrophages is kinetically, immunologically, and physically indistinguishable from rat liver cytosolic sEH. Cytosol from rat liver or inflammatory cells and recombinant rat sEH were incubated with trans-diphenylpropene oxide (tDPPO), a selective substrate for sEH. The tDPPO hydration activity we observed in inflammatory cell cytosol was lower than that from liver. The Km for tDPPO hydration observed in rat inflammatory cell cytosol was the same as the Km for rat liver cytosol (10 microM). Recombinant rat sEH and cytosol from rat liver or inflammatory cells were incubated with the sEH inhibitors, chalcone oxide, 4-fluorochalcone oxide, and 4-phenylchalcone oxide. The IC50 values were 40, 8, and 0.4 microM, respectively, in all samples. Furthermore, sEH activity could be completely immunoprecipitated out of the samples, and the amount of antibody required to do so was apparently identical, regardless of the source of enzyme. SDS-polyacrylamide gel electrophoresis followed by Western blot analysis revealed a single sEH band with a molecular weight of 62 kDa. Isoelectric focusing followed by Western blot analysis revealed multiple bands containing tDPPO-hydrating activity. Although the inflammatory cell bands had the same pattern as those from liver cytosol, the recombinant sEH showed a different banding pattern. These multiple bands were not an artifact of the IEF gel selected. Furthermore, in a 2-dimensional IEF gel, the bands re-migrated to the same position. The presence of sEH in inflammatory cells suggests that this enzyme may have an important endogenous function.

Drumm K, Messner C, Kienast K. **Reactive oxygen intermediate-release of fibre-exposed monocytes increases inflammatory cytokine-mRNA level, protein tyrosine kinase and NF-kappaB activity in co-cultured bronchial epithelial cells (BEAS-2B).** Eur J Med Res 1999;4(7):257-63.
Some pulmonary diseases like bronchitis or asthma bronchiale are mediated by inflammatory mechanisms in bronchial epithelial cells. Alveolar macrophages are located directly in the surrounding of these cells, so that we suppose an interaction between epithelial cells and macrophages regarding to the release of inflammatory mediators. For measuring the contribution of macrophages to the release of inflammatory mediators by bronchial epithelial cells, we established an in vitro model of co-cultured blood monocytes (BM) and BEAS-2B cells in a transwell system (Costar). BM were exposed to Chrysotile B and soot particle FR 101 in a concentration of 100 microg/10(6) cells. After up to 90 min exposure time ELISA, EMSA (electromobility shift assay) and RT-PCR were used to measure protein tyrosine kinase activity, protein activity of NF-kappaB and cytokine (IL-1beta, IL-6, TNF-alpha) specific mRNA levels in BEAS-2B cells. We observed an increase in protein tyrosine kinase activity (up to 1.8 +/- 0.5-fold) and NF-kappaB protein activity in BEAS-2B cells after particle or fibre exposure of co-cultured BM. Consecutive IL-1beta-, IL-6- and TNF-alpha-mRNA were elevated (up to 1.9 +/- 0.58-fold). Protein tyrosine kinase activity, NF-kappaB activity, and the synthesis of cytokine-specific mRNA were inhibited by antioxidants. These data suggest a ROI-dependent NF-kappaB mediated transcription of inflammatory cytokines in bronchial epithelial cells.

Dunzendorfer S, Herold M, Wiedermann CJ. **Inducer-specific bidirectional regulation of endothelial interleukin-8 production by thalidomide.** Immunopharmacology 1999;43(1):59-64.

Interleukin-8 (IL-8) is a potent neutrophil chemotaxin, which can also be produced by endothelial cells to facilitate leukocyte emigration. The aim of this study was to determine the effects of the anti-inflammatory drug thalidomide (THD) on chemotaxin release from endothelial cells. Human umbilical vein endothelial cells (HUVEC) were stimulated with tumor necrosis factor alpha (TNFalpha) or endotoxin (LPS) in the presence or absence of various concentrations of THD. Endothelium-derived interleukin-8 (eIL-8) in supernatants was measured using an enzyme-linked immunosorbent assay (ELISA) and biological activity of the harvested eIL-8 was tested in Boyden chamber chemotaxis assays on PMNL. THD itself had no effect on eIL-8 release. Upon stimulation with TNFalpha or LPS, HUVEC produced increased amounts of eIL-8 and THD affected this process in a bidirectional manner, with augmentation of TNFalpha- and inhibition of LPS-effects. Functionality of eIL-8 was confirmed in chemotaxis experiments and by inhibition of chemotactic effects of supernatants with anti-human IL-8 monoclonal antibodies. Results explain and emphasize immunomodulatory properties of THD in cytokine- and endotoxin-induced inflammation and regulation of transendothelial migration.

Ehlers RA, Hernandez A, Bloemendal LS, Ethridge RT, Farrow B, Evers BM. **Mitochondrial DNA damage and altered membrane potential (delta psi) in pancreatic acinar cells induced by reactive oxygen species.** Surgery 1999;126(2):148-55.
BACKGROUND: Reactive oxygen species (ROS) have been implicated in the induction of acute pancreatitis. Mitochondria possess a distinct genome (mtDNA) that is more susceptible to ROS-induced damage than nuclear DNA (nDNA). The purpose of our study was to determine the effect of ROS on mitochondrial function and membrane potential (delta psi mt), to identify signal transduction mechanisms activated by ROS, and to quantify damage to mtDNA in an in vitro pancreatitis model. METHODS: Pancreatic acinar cells, AR4-2J, were treated with saline solution (control) or hydrogen peroxide (H2O2), a representative ROS. Mitochondrial function was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; to determine delta psi mt, rhodamine-123 uptake was measured. Intracellular calcium levels and c-Jun N-terminal kinase activity was determined; gel mobility shift assays were performed to assess induction of the transcription factor NF-kappa B. To quantitate DNA damage, a novel polymerase chain reaction-based procedure was performed. RESULTS: Mitochondrial function and delta psi mt were significantly decreased with oxidative damage. H2O2 treatment resulted in increased intracellular calcium levels, activation of c-Jun N-terminal kinase, and induction of NF-kappa B DNA binding. Treatment of AR4-2J cells with H2O2 resulted in selective mtDNA damage; nDNA was not affected. CONCLUSIONS: Our data demonstrate that pancreatic mtDNA is more susceptible to oxidative damage than nDNA; this damage is associated with decreases in mitochondrial function and delta psi mt and activation of downstream signal transduction pathways. Mitochondrial damage mediated by ROS may play a central role in pancreatic cell injury associated with acute pancreatitis.

Eichman JD, Robinson JR. **Mechanistic studies on effervescent-induced permeability enhancement**. Pharm Res 1998 Jun;15:925-30.
IPA COPYRIGHT: ASHP The mechanism by which effervescence induces penetration enhancement of a broad range of compounds ranging in size, structure, and other physicochemical properties across rat and rabbit small intestinal epithelium was studied in vitro using a modified Ussing chamber diffusion cell apparatus and in vivo by single-pass intestinal perfusion. Carbon dioxide (CO2) bubbling directly

onto rabbit ileum epithelium induced an increase in drug permeability. Mechanistic studies indicated that effects due to CO2 bubble evolution did not contribute to increases in drug flux. CO2 bubbling induced a reduction in transepithelial electrical resistance indicating epithelial disruption due to a structural change of the paracellular pathway. This was further substantiated by a molecular weight dependence on paracellular marker flux. Tissue recovery was relatively rapid.

Engler KH, Coker R, Evans IH. **A novel colorimetric yeast bioassay for detecting trichothecene mycotoxins.** J Microbiol Methods 1999;35(3):207-18.
A novel colorimetric microbial bioassay for toxicity has been developed; it shows particular sensitivity to trichothecene mycotoxins. The assay uses inhibition of expression of beta-galactosidase activity within the yeast Kluyveromyces marxianus as a sensitive toxicity indicator, cultures remaining yellow, rather than turning deep green-blue, in the presence of X-gal, a chromogenic substrate. The assay is conducted in standard microtitre plates, permitting small volumes (160 microl) and many replicates, and can be scored either automatically by a plate-reader, or by eye. Factors likely to affect the efficacy of the bioassay, including carbon source, solvents, inoculum cell density, and the use of membrane-modulating agents (MMAs), were assessed. Polymyxin B nonapeptide was the most effective toxicity-enhancing MMA tested, enabling the trichothecene mycotoxin, verrucarin A, to be detected at a concentration of about 1 ng/ml. The assay's reproducibility was examined using polymyxin B sulfate, a cheaper MMA, and another trichothecene mycotoxin, T2 toxin: reproducibility and sensitivity were better for the beta-galactosidase X-gal endpoint than for an alternative chromogenic toxicity indicator, the respiratory substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

Eriksson T, Bjorkman S, Roth B, Fyge A, Hoglund P. **Enantiomers of thalidomide: blood distribution and the influence of serum albumin on chiral inversion and hydrolysis.** Chirality 1998;10(3):223-8.
The aim of this investigation was to elucidate the distribution and reactions of the enantiomers of thalidomide at their main site of biotransformation in vivo, i.e., in human blood. Plasma protein binding, erythrocyte: plasma distribution, and the kinetics of chiral inversion and degradation in buffer, plasma, and solutions of human serum albumin (HSA) were studied by means of a stereospecific HPLC assay. The enantiomers of thalidomide were not extensively bound to blood or plasma components. The geometric mean plasma protein binding was 55% and 66%, respectively, for (+)-(R)- and (-)-(S)-thalidomide. The corresponding geometric mean blood:plasma concentration ratios were 0.86 and 0.95 (at a haematocrit of 0.37) and erythrocyte:plasma distributions were 0.58 and 0.87. The rates of inversion and hydrolysis of the enantiomers increased with pH over the range 7.0-7.5. HSA, and to a lesser extent human plasma, catalysed the chiral inversion, but not the degradation, of (+)-(R)- and (-)-(S)-thalidomide. The addition of capric acid or preincubation of HSA with acetylsalicylic acid or physostigmine impaired the catalysis to varying extents. Correction for distribution in blood enhances previously observed differences between the pharmacokinetics of the enantiomers in vivo. The findings also support the notion that chiral inversion in vivo takes place mainly in the circulation and in albumin-rich extravascular spaces while hydrolysis occurs more uniformly in the body. In addition, the chiral inversion and hydrolysis of thalidomide apparently occur by several different mechanisms.

Estacion M, Sinkins WG, Schilling WP. **Stimulation of Drosophila TrpL by capacitative Ca2+ entry.** Biochem J 1999;341(Pt 1):41-9.
Trp-like protein (TrpL, where Trp is transient receptor-potential protein) of Drosophila, a non-selective

cation channel activated in photoreceptor cells by a phospholipase C-dependent mechanism, is thought to be a prototypical receptor-activated channel. Our previous studies showed that TrpL channels are not activated by depletion of internal Ca2+ stores when expressed in Sf9 cells. Using fura-2 to measure cation influx via TrpL, and cell-attached patch recordings to monitor TrpL single-channel activity directly, we have found a thapsigargin-induced increase in TrpL activity in the presence of extracellular bivalent cations, with Ca2+>Sr2+>> Ba2+. The increase in TrpL channel activity was blocked by concentrations of La3+ that completely inhibited endogenous capacitative Ca2+ entry (CCE), but have no effect on TrpL, suggesting that TrpL exhibits trans-stimulation by cation entry via CCE. TrpL has two putative calmodulin (CaM)-binding domains, designated CBS-1 and CBS-2. To determine which site may be required for stimulation of TrpL by the cytosolic free Ca2+ concentration ([Ca2+]i), a chimaeric construct was created in which the C-terminal domain of TrpL containing CBS-2 was attached to human TrpC1, a short homologue of Trp that is not activated by depletion of internal Ca2+ stores or by a rise in [Ca2+]i. This gain-of-function mutant, designated TrpC1-TrpL, exhibited trans-stimulation by Ca2+ entry via CCE. Examination of CaM binding in gel-overlay experiments showed that TrpL and the TrpC1-TrpL chimaera bound CaM, but TrpC1 or a truncated version of TrpL lacking CBS-2 did not. These results suggest that only CBS-2 binds CaM in native TrpL and that the C-terminal domain containing this site is important for trans-stimulation of TrpL by CCE.

Eyer F, Eyer P. **Enzyme-based assay for quantification of paraoxon in blood of parathion poisoned patients.** Hum Exp Toxicol 1998;17(12):645-51.
1. Paraoxon concentration was estimated by means of inhibition kinetics observed with electric eel acetylcholinesterase (AChE) which was determined by a modified Ellman procedure. In human plasma, paraoxon was stabilized by inactivation of paraoxonase with EDTA and aluminon and by inhibition of butyrylcholinesterase with ethopropazine. Paraoxon (1-50 ng) was recovered at 86+/-1.7% (mean+/-s.e. m.) in ether extracts from 0.5 ml samples of spiked stabilized plasma. It could be stored without loss at -20 degrees C for at least 1 month. 2. The enzyme-based assay was applied to follow the paraoxon plasma concentrations in three suicidal patients with severe parathion poisoning. In poisoning with excessive doses and initial paraoxon concentrations above 500 nM, therapeutic obidoxime concentrations of approximately 10 microM failed to essentially reactivate erythrocyte AChE in vivo, while reactivatability ex vivo was nearly complete. With the plasma concentrations of paraoxon dropping below 100 nM, however, reactivation by obidoxime became significant. Unexpectedly, paraoxon levels occasionally reincreased during treatment and resulted in re-inhibition of AChE, bearing some resemblance to the Intermediate Syndrome. 3. The paraoxon concentrations measured fitted satisfactorily the values calculated from the kinetic constants previously obtained for AChE inhibition and obidoxime-induced reactivation in vitro. This indicates that diethylphosphoryloxime formation during obidoxime-induced reactivation does not markedly contribute to the re-inhibition of AChE as observed in vitro.

Fagan JM, Sleczka BG, Sohar I. **Quantitation of oxidative damage to tissue proteins.** Int J Biochem Cell Biol 1999;31(7):751-7.
Active oxygen species are thought to be involved in many physiological and pathological processes and are known to oxidatively modify DNA, lipids and proteins. One such modification is the addition of carbonyl groups to amino acid residues in proteins. The number of carbonyl groups on proteins can be

quantitated spectrophotometrically using 2,4-dinitrophenylhydrazine (DNPH). The DNPH assay described in the literature was found to be unreliable in samples containing high amounts of chromophore (e.g. hemoglobin, myoglobin, retinoids). By using an HCl-acetone wash, hemes from the chromophores could be extracted, enabling the determination of carbonyl content to be made even in highly colored tissue extracts. Residual DNPH, which was also found to interfere with the assay, was removed by additional washes with trichloroacetic acid and ethanol-ethylacetate. These improvements are known to remove lipids, do not lengthen the time required to do the assay, permit quantification of carbonyl content in 1-4 mg protein from a variety of tissue types and provide a sensitive and reliable method for assessing oxidative damage to tissue proteins.

Flores-Murrieta FJ, Ko HC, Flores-Acevedo DM, Lopez-Munoz FJ, Jusko WJ, Sale ME, Castaneda-Hernandez G. **Pharmacokinetic-pharmacodynamic modeling of tolmetin antinociceptive effect in the rat using an indirect response model: a population approach.** J Pharmacokinet Biopharm 1998;26 (5):547-57.
The relationship between the pharmacokinetics and the antinociceptive effect of tolmetin was characterized by an indirect model using a population approach. Animals received an intra-articular injection of uric acid in the right hindlimb to induce its dysfunction. Once dysfunction was complete, rats received an oral tolmetin dose of 1, 3.2, 10, 31.6, 56.2 or 100 mg/kg and antinociceptive effect and blood tolmetin concentration were simultaneously evaluated. Tolmetin produced a dose-dependent recovery of functionality, which was not directly related to blood concentration. An inhibitory indirect response model was used based on these response patterns and the fact that tolmetin reduced nociception by inhibiting prostaglandin synthesis. Pharmacokinetic (PK) and pharmacodynamic (PD) data were simultaneously fitted using nonlinear mixed effects modeling (NONMEM) to the one-compartment model and indirect response model. The individual time courses of the response were described using Bayesian analysis with population parameters as a priori estimates. There was good agreement between the predicted and observed data. Population analysis yielded a maximal inhibition of the nociceptive response of 76% and an IC50 of 9.22 micrograms/ml. This IC50 is similar to that for tolmetin-induced prostaglandin synthesis inhibition in vitro (3.0 micrograms/ml). The present results demonstrate that mechanism-based PK-PD analysis using a population approach is useful for quantitating individual responses as well as reflecting the actual mechanism of action of a given drug in vivo.

Fortini P, Parlanti E, Sidorkina OM, Laval J, Dogliotti E. **The type of DNA glycosylase determines the base excision repair pathway in mammalian cells.** J Biol Chem 1999;274(21):15230-6.
The base excision repair (BER) of modified nucleotides is initiated by damage-specific DNA glycosylases. The repair of the resulting apurinic/apyrimidinic site involves the replacement of either a single nucleotide (short patch BER) or of several nucleotides (long patch BER). The mechanism that controls the selection of either BER pathway is unknown. We tested the hypothesis that the type of base damage present on DNA, by determining the specific DNA glycosylase in charge of its excision, drives the repair of the resulting abasic site intermediate to either BER branch. In mammalian cells hypoxanthine (HX) and 1,N6-ethenoadenine (epsilonA) are both substrates for the monofunctional 3-methyladenine DNA glycosylase, the ANPG protein, whereas 7,8-dihydro-8-oxoguanine (8-oxoG) is removed by the bifunctional DNA glycosylase/beta-lyase 8-oxoG-DNA gly- cosylase (OGG1). Circular plasmid molecules containing a single HX, epsilonA, or 8-oxoG were constructed. In vitro repair assays

with HeLa cell extracts revealed that HX and epsilonA are repaired via both short and long patch BER, whereas 8-oxoG is repaired mainly via the short patch pathway. The preferential repair of 8-oxoG by short patch BER was confirmed by the low efficiency of repair of this lesion by DNA polymerase beta-deficient mouse cells as compared with their wild-type counterpart. These data fit into a model where the intrinsic properties of the DNA glycosylase that recognizes the lesion selects the branch of BER that will restore the intact DNA template.

Foy BD, Toxopeus C, Frazier JM. **Kinetic modeling of slow dissociation of bromosulphophthalein from albumin in perfused rat liver: toxicological implications.** Toxicol Sci 1999;50(1):20-9.
Due to strong binding between organic anions and albumin, the kinetics of the binding process must be carefully considered in biologically-based models used for predictive toxicology applications. Specifically, the slow dissociation rate of an organic anion from the protein may lead to reduced availability of free anion in its flow through the capillaries of an organ. In this work, the effect of the dissociation rate of the anion bromosulphophthalein (BSP) from albumin was studied in isolated, perfused rat livers in the presence of albumin concentrations of 0.25, 1, and 4% (w/v) and an initial BSP concentration of 20 microM. The uptake of BSP from the perfusion medium was modeled using a biologically-based kinetic model of the sinusoidal and intracellular liver compartments. The best fit of the model to data resulted in the prediction of a slow dissociation rate constant for the BSP-albumin of between 0.097 and 0.133 s(-1). Assuming BSP and albumin to be in binding equilibrium in the sinusoidal space, with rapid binding-rate constants, as is often done, produced an unacceptable fit. These results indicate that the strong binding interaction between BSP and albumin, beyond keeping the concentration of free chemical low due to a small equilibrium dissociation constant, can also reduce uptake by an organ due to the slow release of BSP from the protein during passage through the capillaries. The implication of this dissociation-limited condition, when extrapolating to other doses and in-vivo situations, is discussed.

Fuchs WS, Jakobs R, Grunenberg P, Von Nieciecki A, Stanislaus F, et al. **[Influence of different variables on the in vitro dissolution of a sustained-release theophylline dosage form]**. Arzneim Forsch 1998;48(5 Suppl A):552-6. (Ger)
IPA COPYRIGHT: ASHP The effects of pH, osmolarity, rotation speed, and surfactants on the dissolution of a sustained-release dosage form of theophylline anhydrous (Bronchoretard) were studied in vitro. Dissolution was only slightly affected by pH, osmolarity, and stirring speed, and was always within in vivo verified dissolution limits. The addition of sodium lauryl sulfate (sodium dodecyl sulfate) to the dissolution medium markedly altered the dissolution rate; the addition of physiologically surface active human bile did not change the dissolution rate. Comparison with in vivo results indicated that only a physiologically adapted model guarantees reliable results; the addition of synthetic surfactants does not allow for the prediction of bile or food effects.

Furman C, Martin-Nizard F, Fruchart JC, Duriez P, Teissier E. **Differential toxicities of air (mO-LDL) or copper-oxidized LDLs (Cu-LDL) toward endothelial cells.** J Biochem Mol Toxicol 1999;13 (6):316-23.
In vivo low density protein (LDL) oxidation is a progressive phenomenon leading to the presence of minimally and highly oxidized LDLs in the subendothelial arterial space. Oxidized LDLs have been reported to be cytotoxic against endothelial cells. The goal of this study was to determine which of the

minimally and highly oxidized LDLs were the most cytotoxic against bovine aortic endothelial cells (BAEC). Both the morphological aspect of the cells themselves, and LDH or MTT tests revealed that mO- or Cu-LDLs had similar cytotoxicity with up to 8 hours of oxidation, showing no relation with the level of LDL oxidation; for longer oxidation times, Cu-LDL cytotoxicity decreased. This phenomenon is linked to their different oxidation kinetics. Moreover, in the initial hours following BAEC incubation with mO- or Cu-LDLs, total cell glutathione dropped, whereas after 16 hours of incubation, highly oxidized Cu-LDL increased the glutathione level in the cell. The biphasic evolution of glutathione concentration corresponds to an autoprotective mechanism of cells against oxidized LDL cytotoxicity. This study suggests that the specific chemical characteristics of the different types of oxidized LDLs should always be precisely described in future assays devoted to studying the biological effects of what are known under the generic term as "oxidized LDLs". This precaution should prevent any confusion in interpreting different studies.

Galvez AF, De Lumen BO. **A soybean cDNA encoding a chromatin-binding peptide inhibits mitosis of mammalian cells.** Nat Biotechnol 1999;17(5):495-500.
A soybean cDNA encoding the small subunit peptide of a cotyledon-specific 2S albumin (Gm2S-1) is thought to play a role in arresting mitosis during the DNA endoreduplication and cell expansion phase of seed development. The peptide (termed lunasin) contains the cell adhesion motif Arg-Gly-Asp (RGD) followed by eight aspartic acid residues at its C-terminal end. A chimeric gene encoding the lunasin peptide tagged with green fluorescent protein (GFP) arrested cell division, caused abnormal spindle fiber elongation, chromosomal fragmentation, and cell lysis when transiently transfected into murine embryo fibroblast, murine hepatoma, and human breast cancer cells. Deletion of the polyaspartyl end abolished the antimitotic effect. Subcellular localization of lunasin and immunobinding assay using synthetic peptides revealed the preferential adherence of lunasin to chromatin. Immunofluorescence showed that kinetochore proteins were displaced from the centromere in lunasin-transfected cells. These observations suggest that lunasin binds to the chromatin, leading to disruption of kinetochore formation and inhibition of mitosis.

Garberg P, Eriksson P, Schipper N, Sjostrom B. **Automated absorption assessment using Caco-2 cells cultured on both sides of polycarbonate membranes.** Pharm Res 1999;16(3):441-5.
PURPOSE: To increase the capacity of in vitro absorption assessment and to decrease the amount of substance needed to perform early mechanistic investigations. METHODS: A liquid handling system, combined with a shaker and heating plates, was used to automate the Caco-2 cell based in vitro absorption assessment assay. In order to decrease the amount of substance needed for early mechanistic studies, a method for culturing Caco-2 cells on the lower side of polycarbonate membranes was also developed. RESULTS: Similar results were obtained with the automated assay as compared to manually performed assays. Data presented suggest that active transport and efflux were decreased in cells cultured on the lower side of the membranes as compared to ordinary seeded cells. CONCLUSIONS: Implementation of a liquid handling system for in vitro absorption assessment as reported here decrease the manual workload and increases the capacity of this in vitro assay substantially. Caco-2 cells cultured on the lower side of polycarbonate membranes, as described in this article, can not be used for analysis of transport mechanisms.

Goering PL, Thomas D, Rojko JL, Lucas AD. **Mercuric chloride-induced apoptosis is dependent on**

protein synthesis. Toxicol Lett 1999;105(3):183-95.
Apoptosis is a mode of cell death with morphologic and biochemical features that distinguish it from necrosis. Recent studies demonstrating that mercury compounds initiate apoptosis in cultured cells did not elucidate if the biochemical mechanism of apoptosis involved a dependence on macromolecular synthesis post-insult, i.e. programmed cell death. The objectives of this in vitro study were (1) to determine if HgCl2 cytotoxicity includes an apoptotic component, and (2) to determine if apoptosis is dependent on protein synthesis, i.e. proceeds by an inducible mechanism. Suspensions of mouse lymphoma (L5178Y-R) cells were exposed to 0, 1, 5, or 10 microM HgCl2 and apoptosis was evaluated utilizing qualitative and quantitative methods. At 24 h after exposure, transmission electron microscopy revealed a concentration-related increase in morphologic changes typical of apoptosis: margination of condensed chromatin to the nuclear membrane, dilation of the rough endoplasmic reticulum, cytoplasmic condensation and vacuolation, nuclear dissolution, and plasma membrane blebbing. An increase in Hg-induced DNA fragmentation (DNA 'ladder') was observed using agarose gel electrophoresis. Time- and concentration-dependent increases in the percent of apoptotic cells were observed at 1, 6, 12, and 24 h after HgCl2 exposure using a flow cytometric method that discriminates between cells according to size and granularity. Pretreatment of cells with cycloheximide (CHX), an inhibitor of translation, prior to HgCl2 exposure resulted in a 25-50% reduction in apoptotic cells 24 h after exposure to 10 and 20 microM HgCl2, and concomitantly reduced the overall cytotoxicity compared to HgCl2 alone. These results, although limited to a single cell line, support the hypothesis that HgCl2 induces apoptosis that is dependent, at least in part, upon protein synthesis.

Gonzalez MI, Lopez-Colom AM, Ortega A. **Sodium-dependent glutamate transport in Muller glial cells: regulation by phorbol esters.** Brain Res 1999;831(1-2):140-5.
The regulation of the Na(+)-dependent high affinity glutamate/aspartate transporter system expressed in cultured Muller glia cells from chick retina was studied. Treatment of the cells with the Ca(2+)/ diacylglycerol dependent protein kinase C (PKC) activator, phorbol 12-tetradecanoil-13-acetate (TPA) produced a decrease in [(3)H]D-aspartate uptake which was reversed by staurosporine and partially by H7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochoride], two PKC inhibitors. Long-term treatment with TPA resulted in a drastic decrease in the uptake activity, correlated with a substantial fall in the expression of the transporter protein. These findings suggest that PKC is involved in transport modulation at two different levels: phosphorylation and transporter expression in retinal Muller glial cells. Copyright 1999 Elsevier Science B.V.

Gowrishankar TR, Herndon TO, Vaughan TE, Weaver JC. **Spatially constrained localized transport regions due to skin electroporation**. J Controlled Release 1999 Jun 28;57:101-10.
IPA COPYRIGHT: ASHP Localized transport regions in the skin created by electroporation were studied in vitro using model fluorescent molecules. The regions could be constrained to occur at specific sites using electrically insulating masks that restrict the field lines. The increase in total ionic and molecular transport per area was comparable to levels observed in unconstrained electroporation of skin. Constraining the area of intervention to encompass small areas of interest provided the same levels of flux as the unconstrained case.

Gozzi P, Pahlman I, Palmer L, Gronberg A, Persson S. **Pharmacokinetic-pharmacodynamic modeling of the immunomodulating agent susalimod and experimentally induced tumor necrosis factor-**

alpha levels in the mouse. J Pharmacol Exp Ther 1999;291(1):199-203.
The main objective of this study was to explore the concentration-effect relationship between the immunomodulating agent susalimod and lipopolycaccharide (LPS)-induced elevated serum levels of the proinflammatory cytokine tumor necrosis factor-alpha (TNF-alpha). Bacterial LPS (1 mg/kg) was given i.p. along with different doses of susalimod (0, 25, 50, 100, and 200 mg/kg) to female CD-1 mice. Blood samples were drawn at different time points (15-300 min), and serum was analyzed with respect to susalimod and TNF-alpha. The concentration-effect relationship was explored by modeling the data from all dose levels simultaneously using specially written program models, i.e., a three-compartment pharmacokinetic model, including biliary excretion, and an indirect mechanistically based pharmacodynamic model. The models, which were successfully fitted to the experimental data, showed that LPS induced the TNF-alpha synthesis during approximately 70 min and that during this time course, the synthesis rate was governed by the serum phamacokinetics of susalimod. Because the results supported the assumption that the maximum inhibitory effect was equal to full inhibition of the synthesis, the in vivo potency (IC(50)) of susalimod could be estimated to 293 microM. In conclusion, susalimod decreased the LPS-induced TNF-alpha mouse serum levels in a concentration-related manner. The compound is suggested to inhibit the synthesis of TNF-alpha. The integrated pharmacokinetic-pharmacodynamic model estimated the in vivo potency of susalimod in the mouse to be 293 microM.

Haddad S, Tardif R, Viau C, Krishnan K. **A modeling approach to account for toxicokinetic interactions in the calculation of biological hazard index for chemical mixtures.** Toxicol Lett 1999;108(2-3):303-8.
Biological hazard index (BHI) is defined as biological level tolerable for exposure to mixture, and is calculated by an equation similar to the conventional hazard index. The BHI calculation, at the present time, is advocated for use in situations where toxicokinetic interactions do not occur among mixture constituents. The objective of this study was to develop an approach for calculating interactions-based BHI for chemical mixtures. The approach consisted of simulating the concentration of exposure indicator in the biological matrix of choice (e.g. venous blood) for each component of the mixture to which workers are exposed and then comparing these to the established BEI values, for calculating the BHI. The simulation of biomarker concentrations was performed using a physiologically-based toxicokinetic (PBTK) model which accounted for the mechanism of interactions among all mixture components (e.g. competitive inhibition). The usefulness of the present approach is illustrated by calculating BHI for varying ambient concentrations of a mixture of three chemicals (toluene (5-40 ppm), m-xylene (10-50 ppm), and ethylbenzene (10-50 ppm)). The results show that the interactions-based BHI can be greater or smaller than that calculated on the basis of additivity principle, particularly at high exposure concentrations. At lower exposure concentrations (e.g. 20 ppm each of toluene, m-xylene and ethylbenzene), the BHI values obtained using the conventional methodology are similar to the interactions-based methodology, confirming that the consequences of competitive inhibition are negligible at lower concentrations. The advantage of the PBTK model-based methodology developed in this study relates to the fact that, the concentrations of individual chemicals in mixtures that will not result in a significant increase in the BHI (i.e. > 1) can be determined by iterative simulation.

Hayakawa N, Nozawa K, Ogawa A, Kato N, Yoshida K, Akamatsu KI, Tsuchiya M, Nagasaka A, Yoshida S. **Isothiazolone derivatives selectively inhibit telomerase from human and rat cancer cells**

in vitro. Biochemistry 1999;38(35):11501-7.
The telomere hypothesis postulates stabilization of telomere length and telomerase activation as key events in cellular immortalization and carcinogeneses. Accordingly, telomerase has been suggested as a novel and highly selective target for design of antitumor drugs. Screening of a chemical library including 16 000 synthetic compounds yielded six that strongly inhibited telomerase activity in extracts of cultured human cells, including four isothiazolone derivatives and two unrelated compounds. The most potent inhibitor was 2-[3-(trifluoromethyl)phenyl]isothiazolin-3-one (TMPI), a concentration of 1.0 microM inhibited telomerase activity by 50% according to a telomere repeat amplification protocol (TRAP) assay. Analysis using partially purified telomerase from AH7974 rat hepatoma cells demonstrated noncompetitive inhibition with the telomere-repeat primer and mixed inhibition with the dNTPs; the inhibition constant was 2.5 microM. TMPI did not inhibit eukaryotic DNA polymerase alpha, beta, or human immunodeficiency virus reverse transcriptase (HIV RT). Thus, inhibition by TMPI was highly selective for telomerase. Inhibition by TMPI was quenched by 1 mM of dithiothreitol or glutathione, suggesting that TMPI inhibits telomerase by acting at a cysteine residue. TMPI inhibition of this enzyme may find application as an antineoplastic agent.

Hayashi Y, Matsumoto K. **[Toxicity evaluation of pharmaceuticals and mechanisms of their effects]**. Nippon Yakurigaku Zasshi 1999;113(1):19-30. (Jpn)
Preclinical studies are defined as experiments other than clinical trials that are conducted in test systems under laboratory conditions for determining the safety of test materials for anticipated human use. It is known that preclinical studies are an indispensable requisite for but constitute time/resource-consuming processes in research and development of new pharmaceuticals. Therefore, they must be designed and conducted in a manner to satisfy the criteria that the obtained data are mutually acceptable among various countries to avoid unnecessary duplication of testing. The purpose of the International Conference on Harmonisation (ICH) has been directed towards the resolution of this issue. Major scientific issues in preclinical studies comprise the interpretation of test data with respect to prediction of potential adverse effects of a test material in humans. Mechanistic consideration of the toxic effects occurring in animals given a test material can usually provide a scientific basis for evaluating the potential hazard of the material in humans. For example, when a test material was found to exhibit a carcinogenic effect in a long-term animal test, one will attempt to determine, on the basis of data from genotoxicity studies, repeated dose toxicity tests, toxicokinetic studies or pharmacology studies on the substance, whether the carcinogenic effect is due to its genotoxicity (genotoxic carcinogen) or a sequela of some secondary mechanisms (non-genotoxic carcinogen). In the cases where a test material was shown to exert toxic effects with either functional manifestation or non-neoplastic morphological manifestation, elucidation of the mechanism will also be useful for extrapolation of animal data to the human situation. It is known that pharmaceuticals induce their toxic effects through various mechanisms such as covalent binding of active intermediates with macrolecules of target cells, oxidative stress-mediated effects, hormone-mediated effects or cytokinemediated effects. As shown in hepatocarcinogenesis or elevation of plasma transaminase activities in rodents attributable to activation of PPAR-alpha, nuclear receptors or ligand-dependent transcription factors are, now, regarded as important targets for toxicity evaluation of pharmaceuticals.

Hayes JS, Lawler OA, Walsh MT, Kinsella BT. **The prostacyclin receptor is isoprenylated.**

Isoprenylation is required for efficient receptor-effector coupling. J Biol Chem 1999;274(34):23707-18.
The prostacyclin receptor (IP), a G protein-coupled receptor, mediates the actions of the prostanoid prostacyclin and its mimetics. IPs from a number of species each contain identically conserved putative isoprenylation CAAX motifs, each with the sequence CSLC. Metabolic labeling of human embryonic kidney (HEK) 293 cells stably overexpressing the hemagluttinin epitope-tagged IP in the presence of [(3)H]mevalonolactone established that the mouse IP is isoprenylated. Studies involving in vitro assays confirmed that recombinant forms of the human and mouse IP are modified by carbon 15 farnesyl isoprenoids. Disruption of isoprenylation, by site-directed mutagenesis of Cys(414) to Ser(414), within the CAAX motif, abolished isoprenylation of IP(SSLC) both in vitro and in transfected cells. Scatchard analysis of the wild type (IP) and mutant (IP(SSLC)) receptor confirmed that each receptor exhibited high and low affinity binding sites for [(3)H]iloprost, which were not influenced by receptor isoprenylation. Whereas stable cell lines overexpressing IP generated significant agonist (iloprost and cicaprost)-mediated increases in cAMP relative to nontransfected cells, cAMP generation by IP(SSLC) cells was not significantly different from the control, nontransfected HEK 293 cells. Moreover, co-expression of the alpha (alpha) subunit of Gs generated significant augmentations in cAMP by IP but not by IP(SSLC) cells. Whereas IP also demonstrated significant, dose-dependent increases in [Ca(2+)](i) in response to iloprost or cicaprost compared with the nontransfected HEK 293 cells, mobilization of [Ca(2+)](i) by IP(SSLC) was significantly impaired. Co-transfection of cells with either Galpha(q) or Galpha(11) resulted in significant augmentation of agonist-mediated [Ca(2+)](i) mobilization by IP cells but not by IP(SSLC) cells or by the control, HEK 293 cells. In addition, inhibition of isoprenylation by lovastatin treatment significantly reduced agonist-mediated cAMP generation by IP in comparison to the nonisoprenylated beta(2) adrenergic receptor or nontreated cells. Hence, isoprenylation of IP does not influence ligand binding but is required for efficient coupling to the effectors adenylyl cyclase and phospholipase C.

Heidenreich O, Neininger A, Schratt G, Zinck R, Cahill MA, Engel K, Kotlyarov A, Kraft R, Kostka S, Gaestel M, et al. **MAPKAP kinase 2 phosphorylates serum response factor in vitro and in vivo.** J Biol Chem 1999;274(20):14434-43.
Several growth factor- and calcium-regulated kinases such as pp90(rsk) or CaM kinase IV can phosphorylate the transcription factor serum response factor (SRF) at serine 103 (Ser-103). However, it is unknown whether stress-regulated kinases can also phosphorylate SRF. We show that treatment of cells with anisomycin, arsenite, sodium fluoride, or tetrafluoroaluminate induces phosphorylation of SRF at Ser-103 in both HeLa and NIH3T3 cells. This phosphorylation is dependent on the kinase p38/SAPK2 and correlates with the activation of MAPKAP kinase 2 (MK2). MK2 phosphorylates SRF in vitro at Ser-103 with similar efficiency as the small heat shock protein Hsp25 and significantly better than CREB. Comparison of wild type murine fibroblasts with those derived from MK2-deficient mice (Mk(-/-)) reveals MK2 as the major SRF kinase induced by arsenite. These results demonstrate that SRF is targeted by several signal transduction pathways within cells and establishes SRF as a nuclear target for MAPKAP kinase 2.

Heinlein CA, Ting HJ, Yeh S, Chang C. **Identification of ARA70 as a ligand-enhanced coactivator for the peroxisome proliferator-activated receptor gamma.** J Biol Chem 1999;274(23):16147-52.

In an effort to understand transcriptional regulation by the peroxisome proliferator-activated receptor gamma (PPARgamma), we have investigated its potential interaction with coregulators and have identified ARA70 as a ligand-enhanced coactivator. ARA70 was initially described as a coactivator for the androgen receptor (AR) and is expressed in a range of tissues including adipose tissue (Yeh, S., and Chang, C. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5517-5521). Here we show that ARA70 and PPARgamma specifically interact by coimmunoprecipitation and in a mammalian two-hybrid assay. PPARgamma and ARA70 interact in the absence of the PPARgamma ligand 15-deoxy-Delta12,14-prostaglandin J2, although the addition of exogenous ligand enhances this interaction. Similarly, in transient transfection of DU145 cells, cotransfection of PPARgamma and ARA70 induces transcription from reporter constructs driven by either three copies of an isolated PPAR response element or the natural promoter of the adipocyte fatty acid-binding protein 2 in the absence of exogenous 15-deoxy-Delta12,14-prostaglandin J2. However, this PPARgamma-ARA70 transactivation is enhanced by the addition of ligand. Thus, ARA70 can function as a ligand-enhanced coactivator of PPARgamma. Finally, we show that AR can squelch PPARgamma-ARA70 transactivation, which suggests that cross-talk may occur between PPARgamma- and AR-mediated responses in adipocytes.

Hirose M, Takahashi S, Ogawa K, Futakuchi M, Shirai T, Shibutani M, Uneyama C, Toyoda K, Iwata H. **Chemoprevention of heterocyclic amine-induced carcinogenesis by phenolic compounds in rats.** Cancer Lett 1999;143(2):173-8.
Chemopreventive effects of synthetic and naturally occurring antioxidants on heterocyclic amine (HCA)-induced rat carcinogenesis and mechanisms of inhibition were assessed. In a medium-term liver bioassay, combined treatment with 0.03% 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) and synthetic antioxidants such as 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ), BHA, BHT, tert-butylhydroquinone (TBHQ) and propyl gallate, each at a dose of 0.25%, and troglitazone at doses 0.5 and 0.1%, potently inhibited development of glutathione S-transferase placental form (GST-P) positive foci as compared with MeIQx alone values. Of these antioxidants, HTHQ showed the greatest activity. Green tea catechins tended to inhibit GST-P positive foci development, while quercetin, rutin, curcumin, daidzin, ferulic acid and genistin all exerted significant enhancing effects. HTHQ also inhibited 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced colon carcinogenesis in a two stage colon carcinogenesis model using 1,2-dimethylhydrazine (DMH) as an initiator. Immunohistochemically detected PhIP-DNA adduct positive nuclei in the colon induced by continuous oral treatment with 0.02% PhIP for 2 weeks decreased by the combined treatment with 0.5 or 0.125% HTHQ. Methoxyresorfin O-demethylase activity in rat liver microsomes in vitro was clearly inhibited by the addition of HTHQ, BHA, BHT, TBHQ or propyl gallate, with particularly strong inhibition being observed in HTHQ. However, the CYP1A2 level in rat liver increased after oral treatment with HTHQ for 2 weeks. These results indicate that synthetic antioxidants, HTHQ in particular, is a very strong chemopreventor of HCA-induced carcinogenesis. It is suggested that depression of metabolic activation rather than antioxidant activity is responsible for the observed effect. However, other mechanisms, including the effects on phase II enzymes cannot be ruled out.

Hogstrand C, Verbost PM, Wendelaar Bonga SE. **Inhibition of human erythrocyte Ca2+-ATPase by Zn2+.** Toxicology 1999;133(2-3):139-45.
Recent investigations suggest that Ca2(+)-ATPase from fish gills is very sensitive to Zn2+ (Hogstrand et

al., 1996. Am. J. Physiol. 270, R1141-R1147). The effect of free Zn2+ ion on the human erythrocyte plasma membrane Ca2(+)-ATPase was investigated to explore the possible extension of this finding to humans. Membrane vesicles were prepared and the Ca2(+)-ATPase activity was measured as Ca2(+)-stimulated ATP hydrolysis and as ATP-dependent Ca2+ transport. The Zn2+ ion inhibited the erythrocyte Ca2(+)-ATPase by reducing Vmax and increasing the K0.5. While in the Ca2+ transport assay only the Vmax was affected at lower Zn2+ concentrations (50-100 pM), reduction of Vmax was always accompanied by an affinity decrease in the ATP hydrolysis assay. The Ca2(+)-ATPase was found to be inhibited by Zn2+ at extremely low concentrations. The IC10 and IC50 for Zn2+, at a Ca2+ concentration of 1.0 microM, were estimated at 4 and 80 pM, respectively. Although the Ca2(+)-ATPase might be more sensitive in vitro than in vivo conditions, the results suggest that physiological concentrations of Zn2+ may reduce the activity of the erythrocyte Ca2(+)-ATPase. Furthermore, disturbance of Ca homeostasis may be a mechanism causing Zn toxicity during exposure.

Huang Z, Waxman DJ. **High-performance liquid chromatographic-fluorescent method to determine chloroacetaldehyde, a neurotoxic metabolite of the anticancer drug ifosfamide, in plasma and in liver microsomal incubations.** Anal Biochem 1999;273(1):117-25.
Chloroacetaldehyde (CA) is a nephrotoxic and neurotoxic metabolite of the anticancer drug ifosfamide (IFA) and is a dose-limiting factor in IFA-based chemotherapy. Plasma levels of CA in IFA-treated cancer patients are often difficult to determine due to the lack of a sufficiently sensitive and specific analytical method. We have developed a simple and sensitive HPLC method with fluorescence detection to measure CA formation catalyzed by liver cytochrome P450 enzymes, either in vivo in IFA-injected rats or in vitro in liver microsomal incubations. This method is based on the formation of the highly fluorescent adduct 1-N(6)-ethenoadenosine from the reaction of CA with adenosine (10 mM) at pH 4.5 upon heating at 80 degrees C for 2 h. The derivatization mixture is directly injected onto a C18 HPLC column and is monitored with a fluorescence detector. Calibration curves are linear ($r > 0.999$) over a wide range of CA concentrations (5-400 pmol). The limit of detection of CA in plasma using this method is <0.1 microM and only 50 microl of plasma is required for the assay. By coupling this method with a recently described HPLC-fluorescent method to determine acrolein, a cytochrome P450 metabolite of IFA formed during the activation of the drug by 4-hydroxylation, the two major, alternative P450-catalyzed pathways of IFA metabolism can be monitored from the same plasma samples or liver microsomal incubations and the partitioning of drug between these two pathways thereby quantitated. This assay may prove to be useful for studies of IFA metabolism aimed at identifying factors that contribute to individual differences in CA formation and in developing approaches to minimize CA formation while maximizing IFA cytotoxicity. Copyright 1999 Academic Press.

Ide N, Lau BH. **S-Allylcysteine attenuates oxidative stress in endothelial cells**. Drug Dev Ind Pharm 1999;25(5):619-24.
IPA COPYRIGHT: ASHP The antioxidant effects of S-allylcysteine were determined using several in vitro assay systems; pulmonary artery endothelial cells (PAECs) were preincubated with SAC at 37DGC and 5% carbon dioxide for 24 h, washed, and then exposed to 0.1 mg/ml oxidized low-density lipoprotein (LDL) for 24 h. Lactate dehydrogenase (LDH) release and intracellular glutathione (GSH) levels were determined. Oxidized LDL caused an increase of LDH release and depletion of GSH.

Pretreatment with SAC prevented these changes. Peroxides were measured directly in 24-well plates. SAC dose-dependently inhibited oxidized LDL-induced release of peroxides in PAEC. In a cell-free system, SAC was shown to scavenge hydrogen peroxide. It was concluded that SAC can protect endothelial cells from oxidized LDL-induced injury by removing peroxides and preventing intracellular GSH depletion.

Itakura A, Kurauchi O, Morikawa S, Furugori K, Mizutani S, Tomoda Y. **Human amniotic fluid motogenic activity for fetal alveolar type II cells by way of hepatocyte growth factor.** Obstet Gynecol 1997 May;89(5 Pt 1):729-33.
OBJECTIVE: To find out if hepatocyte growth factor (HGF) in amniotic fluid (HGF-AF) has a direct effect on fetal lung development, we investigated the effects of AF as well as recombinant human HGF (rhHGF) on proliferation, migration, and morphogenesis of fetal alveolar type II cells in vitro. METHODS: Amniotic fluid samples were obtained from 37 women at various gestational ages. Mitogenic, motogenic, and morphogenic activity was investigated by 5-bromo-2'-deoxyuridine incorporation, Boyden chamber assay, and culture in collagen-gels, respectively. RESULTS: The motility of AK-D cells was stimulated by AF from 14 to 31 weeks' gestation in proportion to the concentration of HGF-AF, and this effect was comparable to that observed with rhHGF. Furthermore, this activity was neutralized by anti-human HGF antibody. However, AF samples subsequent to 32 weeks had no motogenic influence despite the continued presence of immunoreactive HGF-AF. Neither increased DNA synthesis nor morphogenesis in response to AF was identified under the conditions used. CONCLUSION: The present study suggests that AF stimulates alveolar type II cell migration by way of HGF-AF in vitro.

Jang JY, Droz PO, Chung H. **Uncertainties in physiologically based pharmacokinetic models caused by several input parameters.** Int Arch Occupat Environ Health 1999;72(4);247-54.
BIOSIS COPYRIGHT: BIOL ABS. Objective: One of the problems in the application of physiologically based pharmacokinetic (PB-PK) models is that authors often use different input parameters, with unknown influence on the results. Differences in the simulation results obtained with various sets of parameters are examined herein. Method: Chemicals considered were perchloroethylene, toluene, and styrene. Simulations of alveolar concentrations, blood concentrations, and urinary metabolite excretions were performed for the three s also differed according to the authors. Such differences in input parameter values proved to have a large influence on PB-PK model results and, therefore, increased their uncertainties. Uncertainties were much more significant in urinary metabolite concentration than in alveolar and blood concentration for chemicals that are poorly metabolized. On the other hand, uncertainties were more significant in alveolar and blood concentrations than in urinary metabolite excretions for chemicals that are.

Jaquet V, Pfend G, Tosic M, Matthieu JM. **Analysis of cis-acting sequences from the myelin oligodendrocyte glycoprotein promoter.** J Neurochem 1999;73(1):120-8.
Myelin oligodendrocyte glycoprotein (MOG), a minor component of the myelin sheath, appears to be implicated in the late events of CNS myelinogenesis. To investigate the transcriptional regulation of MOG, 657 bp of the 5'-flanking sequence of the murine MOG gene, previously shown to induce the highest level of transcription in an oligodendroglial cell line, was analyzed by in vitro footprinting and electrophoretic mobility shift assays. This region contains at least three sites that contact nuclear proteins

in vitro. Each region described in this study binds specific nuclear proteins and enhances transcription in the OLN-93 glial cell line. More specifically, a region located at position -93 to -73 bp, which displays 100% homology in mouse and human MOG promoters, presents distinct binding affinities between brain and liver nuclear proteins. The results obtained by supershift assay and site-directed mutagenesis reveal that this region contains an essential positive element (TGACGTGG) related to the cyclic AMP-responsive element CREB-1 and are additional evidence for the involvement of the cyclic AMP transduction pathway in oligodendrocyte development.

Jett DA, Navoa RV, Lyons MA Jr. **Additive inhibitory action of chlorpyrifos and polycyclic aromatic hydrocarbons on acetylcholinesterase activity in vitro.** Toxicol Lett 1999;105(3):223-9.
This study tested the hypothesis that the inhibition of acetylcholinesterase is greater when the insecticide chlorpyrifos (CPF) is in the presence of several polycyclic aromatic hydrocarbons (PAHs) found in house dust. CPF-oxon (CPFO) inhibition curves of purified AChE (electric eel) were generated in the presence or absence of different concentrations of the PAHs pyrene, benzo(a)pyrene, anthracene, and fluoranthene. Without CPF-oxon, all four PAHs themselves inhibited AChE activity with IC50 values in the range 8.2-17 microM. The IC50 for benzo(a)pyrene with human recombinant AChE was 1.5 microM. When AChE was incubated with CPF-oxon together with the PAHs, the inhibitory effect on AChE was additive. This was exemplified by large (60-80%) and significant ($P<0.01$) inhibition in AChE activity by the PAHs when combined with nanomolar concentrations of CPF-oxon. Kinetic studies indicated that benzo(a)pyrene inhibited AChE in a noncompetitive manner, and the reduction in maximal velocity (Vmax) by benzo(a)pyrene and CPFO together was the sum of the inhibitory effect of the two inhibitors alone, further supporting an additive effect. These data suggest that some PAHs have anticholinesterase activity, and contribute in an additive manner to the inhibitory effect of CPFO on AChE in vitro. Further research is needed to determine the toxicological relevance of these findings.

Jewess PJ, Devonshire AL. **Kinetic microplate-based assays for inhibitors of mitochondrial NADH: Ubiquinone oxidoreductase (complex I) and succinate:Cytochrome c oxidoreductase.** Anal Biochem 1999;272(1):56-63.
BIOSIS COPYRIGHT: BIOL ABS. Kinetic microplate-based assays for both mitochondrial NADH: ubiquinone oxidoreductase (complex I) and succinate:cytochrome c oxidoreductase using insect submitochondrial particles as the source of the enzyme activities have been developed. These assays have been used to design high-throughput screens for inhibitors of these mitochondrial electron transfer activities to assess their intrinsic in vitro efficacies as potential pesticides. These methods can be used to test up to 60 compounds per day.

Kapoor TM, Mitchison TJ. **Allele-specific activators and inhibitors for kinesin.** Proc Natl Acad Sci U S A 1999;96(16):9106-11.
Members of the kinesin superfamily are force-generating ATPases that drive movement and influence cytoskeleton organization in cells. Often, more than one kinesin is implicated in a cellular process, and many kinesins are proposed to have overlapping functions. By using conventional kinesin as a model system, we have developed an approach to activate or inhibit a specific kinesin allele in the presence of other similar motor proteins. Modified ATP analogs are described that do not activate either conventional kinesin or another superfamily member, Eg5. However, a kinesin allele with Arg-14 in its nucleotide binding pocket mutated to alanine can use a subset of these nucleotide analogs to drive

microtubule gliding. Cyclopentyl-ATP is one such analog. Cyclopentyl-adenylylimidodiphosphate, a nonhydrolyzable form of this analog, inhibits the mutant allele in microtubule-gliding assays, but not wild-type kinesin or Eg5. We anticipate that the incorporation of kinesin mutants and allele-specific activators and inhibitors in in vitro assays should clarify the role of individual motor proteins in complex cellular processes.

Kawahara M, Nanbo T, Tsuji A. **Physiologically based pharmacokinetic prediction of p-phenylbenzoic acid disposition in the pregnant rat**. Biopharm Drug Dispos 1998 Oct;19:445-53.
IPA COPYRIGHT: ASHP To develop a detailed physiological pharmacokinetic model for tissue distribution and elimination of p-phenylbenzoic acid in the pregnant rat, pregnant rats received intravenous radiolabeled p-phenylbenzoic acid then were killed at 5-720 min after drug administration and maternal blood and tissues and fetal blood and tissues were analyzed for drug concentration; plasma, renal, and nonrenal clearances were measured and transplacental clearance, skin-amniotic fluid clearances, and fetal metabolic clearance were taken from previously reported analysis and a physiologically based pharmacokinetic model was generated. The physiological model successfully predicted the p-phenylbenzoic acid concentration-time profiles for both mother and fetuses.

Kehlenbach RH, Dickmanns A, Kehlenbach A, Guan T, Gerace L. **A role for RanBP1 in the release of CRM1 from the nuclear pore complex in a terminal step of nuclear export.** J Cell Biol 1999;145 (4):645-57.
We recently developed an assay in which nuclear export of the shuttling transcription factor NFAT (nuclear factor of activated T cells) can be reconstituted in permeabilized cells with the GTPase Ran and the nuclear export receptor CRM1. We have now used this assay to identify another export factor. After preincubation of permeabilized cells with a Ran mutant that cannot hydrolyze GTP (RanQ69L), cytosol supports NFAT export, but CRM1 and Ran alone do not. The RanQ69L preincubation leads to accumulation of CRM1 at the cytoplasmic periphery of the nuclear pore complex (NPC) in association with the p62 complex and Can/Nup214. RanGTP-dependent association of CRM1 with these nucleoporins was reconstituted in vitro. By biochemical fractionation and reconstitution, we showed that RanBP1 restores nuclear export after the RanQ69L preincubation. It also stimulates nuclear export in cells that have not been preincubated with RanQ69L. RanBP1 as well as Ran-binding domains of the cytoplasmic nucleoporin RanBP2 promote the release of CRM1 from the NPC. Taken together, our results indicate that RanGTP is important for the targeting of export complexes to the cytoplasmic side of the NPC and that RanBP1 and probably RanBP2 are involved in the dissociation of nuclear export complexes from the NPC in a terminal step of transport.

Keys DA, Wallace DG, Kepler TB, Conolly RB. **Quantitative evaluation of alternative mechanisms of blood and testes disposition of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in rats.** Toxicol Sci 1999;49(2):172-85.
Di(2-ethylhexyl) phthalate (DEHP), a commercially important plasticizer, induces testicular toxicity in laboratory animals at high doses. After oral exposure, most of the DEHP is rapidly metabolized in the gut to mono(2-ethylhexyl) phthalate (MEHP), which is the active metabolite for induction of testicular toxicity. To quantify the testes dose of MEHP with various routes of exposure and dose levels, we developed a physiologically based pharmacokinetic (PBPK) model for DEHP and MEHP in rats. Tissue: blood partition coefficients for DEHP were estimated from the n-octanol: water partition coefficient,

while partition coefficients for MEHP were determined experimentally using a vial equilibration technique. All other parameters were either found in the literature or estimated from blood or tissue levels following oral or intravenous exposure to DEHP or MEHP. A flow-limited model failed to adequately simulate the available data. Alternative plausible mechanisms were explored, including diffusion-limited membrane transport, enterohepatic circulation, and MEHP ionization (pH-trapping model). In the pH-trapping model, only nonionized MEHP is free to become partitioned into the tissues, where it is equilibrated and trapped as ionized MEHP until it is deionized and released. All three alternative models significantly improved predictions of DEHP and MEHP blood concentrations over the flow-limited model predictions. The pH-trapping model gave the best predictions with the largest value of the log likelihood function. Predicted MEHP blood and testes concentrations were compared to measured concentrations in juvenile rats to validate the pH-trapping model. Thus, MEHP ionization may be an important mechanism of MEHP blood and testes disposition in rats.

Kietzmann T, Hirsch-Ernst KI, Kahl GF, Jungermann K. **Mimicry in primary rat hepatocyte cultures of the in vivo perivenous induction by phenobarbital of cytochrome P-450 2B1 mRNA: role of epidermal growth factor and perivenous oxygen tension.** Mol Pharmacol 1999;56(1):46-53.
Treatment of male rats with phenobarbital (PB) results in a perivenous and mid-zonal pattern of cytochrome P-450 (CYP)2B1 mRNA expression within the liver acinus. The mechanism of this zonated induction is still poorly understood. In this study sinusoidal gradients of oxygen and epidermal growth factor (EGF) besides those of the pituitary-dependent hormones growth hormone (GH), thyroxine (T4), and triiodothyronine (T3) were considered to be possible determinants for the zonated induction of the CYP2B1 gene in liver. Moreover, heme proteins seem to play a key role in oxygen sensing. Therefore, the influence of arterial (16% O2) and venous (8% O2) oxygen tension (pO2), and of the heme synthesis inhibitors CoCl2 and desferrioxamine (DSF) on PB-dependent CYP2B1 mRNA induction as well as the repression by EGF and, for comparison, by GH, T4, and T3, of the induction under arterial and venous pO2 were investigated in primary rat hepatocytes. Within 3 days, phenobarbital induced CYP2B1 mRNA to maximal levels under arterial pO2 and to about 40% of maximal levels under venous pO2. CoCl2 annihilated induction by PB under both oxygen tensions, whereas desferrioxamine and heme abolished the positive modulation by O2, suggesting that heme is a necessary component for O2 sensing. EGF suppressed CYP2B1 mRNA induction by PB only under arterial but not under venous pO2, whereas GH, T4, and T3 inhibited induction under both arterial and venous pO2. Thus, in hepatocyte cultures, an O2 gradient in conjunction with EGF mimicked the perivenous induction by PB of the CYP2B1 gene observed in the liver in vivo.

Kishi S, Goto N, Nakamura T, Ueda T. **Evaluation of cell-killing effects of 1-beta-D-arabinofuranosylcytosine and daunorubicin by a new computer-controlled in vitro pharmacokinetic simulation system.** Cancer Res 1999;59(11):2629-34.
An in vitro pharmacokinetic simulation system that can simulate plasma pharmacokinetics was established to evaluate the cytotoxicity of two representative antileukemic agents, 1-beta-D-arabinofuranosylcytosine (ara-C) and daunorubicin. With this system, the survival rate of the cell line K562 treated with ara-C, relative to that of untreated control cells, was 7.1%, as determined by a clonogenic culture technique using a clinically intermediate dose of ara-C (1.0 g/m2; 2-h infusion). When the area under the serum concentration-time curve (AUC) was kept constant at infusion times of

2, 4, 8, and 16 h with a regular dose of ara-C (100-mg/m2 infusion), the relative cell survival rates were 75, 72, 34, and 14%, respectively. In contrast, with the use of a conventional culture system and a constant concentration-time product (C x T), no time-dependent inhibition effect by ara-C was observed, probably due to the ara-C inactivation in the cell-containing culture medium. For example, 93% of the ara-C in the cell-suspended medium in the conventional culture system was converted to its inactive form, 1-beta-D-arabinofuranosyluracil, within 16 h after addition of ara-C to the medium. For the simulations of the administration of 50 mg/m2 daunorubicin for 0.5-, 2-, 4-, and 8-h infusions, the relative survival rates [maximal concentrations (Cmaxs)] were 37.4% (0.24 microM), 49.7% (0.089 microM), 72.1% (0.055 microM), and 82.2% (0.032 microM), respectively. With the conventional culture system, the relative survival rates (Cmaxs) following daunorubicin treatment were 7.6% (0.48 microM), 18.6% (0.12 microM), 63.7% (0.06 microM), and 92.0% (0.03 microM) when the drug exposure times were 0.5, 2, 4, and 8 h, respectively, with a constant C x T. When the drug concentration for 90% cell killing by the conventional culture system was plotted against the exposure time on a logarithmic scale, the regression line for daunorubicin had a slope of -0.40, whereas the slope of cis-diamminedichloroplatinum (or cisplatin), a typical AUC-dependent drug, was -0.98. These results suggested that daunorubicin was Cmax dependent rather than AUC dependent. In the simulation system, this Cmax dependency was apparently reduced, probably because of the smaller difference of Cmaxs in the simulation system compared with the conventional system with the constant AUC. Thus, this simulation system can predict the effects of ara-C, daunorubicin, and other antineoplastic agents much more exactly than the conventional culture system in clinical use.

Kitazawa T, Uchiyama F, Hirose K, Taneike T. **Characterization of the muscarinic receptor subtype that mediates the contractile response of acetylcholine in the swine myometrium.** Eur J Pharmacol 1999;367(2-3):325-34.
The aim of the present study was to characterize the subtype of muscarinic receptor that mediates acetylcholine-induced contractions in the nonpregnant proestrus swine myometrium by means of mechanical, radioligand ([3H]quinuclidinyl benzilate) binding and biochemical (measurement of cyclic AMP) approaches. Acetylcholine (-logEC50, 6.12), oxotremorine-methiodide (6.47), methacholine (6.35), carbachol (6.18) and muscarine (6.33) caused contractile responses of the uterine circular muscle, with a similar maximum amplitude, but pilocarpine and McN-A-343 (4-(m-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium) were ineffective in causing contraction. The contractile response to acetylcholine was antagonized by the following muscarinic receptor antagonists in a competitive manner (with pA2 values in parentheses): atropine (8.95), 4-diphenylacetoxy-N-methylpiperidine (4-DAMP, 8.83), tropicamide (7.07), himbacine (7.01), pirenzepine (6.42) and 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-- 6H-pyri do[2,3 b][1,4]benzodiazepin-6-one (AF-DX116, 5.96). Electrical field stimulation (10 Hz) caused tetrodotoxin- and atropine-sensitive contractions in the circular muscle. All muscarinic receptor antagonists decreased the electrical field stimulation-induced contraction in a concentration-dependent manner. The order of inhibition (-logIC50) was 4-DAMP (8.35) > tropicamide (6.72) > himbacine (6.54) > pirenzepine (6.31)> AF-DX116 (6.13). Acetylcholine did not affect the cytoplasmic cyclic AMP level, regardless of the presence or absence of forskolin, suggesting the absence of functional muscarinic M2 and/or M4 receptors in the swine myometrium. The receptor binding study indicated that circular muscle layers of the swine myometrium contained a single class of [3H]quinuclidinyl benzilate binding site (Kd = 0.92 nM; Bmax = 126.6 fmol/

mg protein). Specific binding was displaced by muscarinic receptor antagonists in the following order (with pKi value and Hill coefficient in parentheses): atropine (8.22 and 0.93) > 4-DAMP (8.18 and 0.94) > tropicamide (6.78 and 0.93) > pirenzepine (5.46 and 0.92) > AF-DX116 (5.12 and 0.94). The present results suggest that in circular muscle layers of the swine myometrium, exogenous and endogenous acetylcholine cause contraction through activation of muscarinic M3 receptors present on smooth muscle cells.

Kohen E, Gatt S, Schachtschabel A, Schachtschabel DO, Kohen C, Agmon V, Hirschberg JG, Monti M, Roisen F. **Multiprobe fluorescence imaging and microspectrofluorimetry of cell transformation and differentiation: implications in terms of applied biochemistry and biotechnology.** Biotechnol Appl Biochem 1999;29(Pt 3):191-205.
The dichotomy of cellular transformation versus differentiation does not preclude the hypothesis of a unified underlying mechanism that can switch either way as a result of growth factors, cell-membrane receptors, secondary messengers, integrating switch kinases and/or nuclear receptors. Its study for biopharmaceutical and biotechnological applications requires a methodology capable of dealing with such pleiotropy. In the multiprobe-multiparameter approach, one must remain wary of cumulative toxic effects and misinterpretations. 'Smart' instrumentation does not mean 'smart' probes. It turns out that the cell's own endogenous probes, the fluorescent coenzymes, may be akin to 'smart' probes, open to study in situ of many-fold interrelated pathways in cell energetics and dynamics. Resolution at the micro- and even nano-compartment levels is not altogether impossible. Thus an innovative search in terms of what may be called 'intracellular reconnaissance with fluorescent probes and biopharmaceuticals' necessitates recourse to multiple tentative probings along the pleiotropic mechanisms as far in resolution as one can go. Among the characteristic findings using this approach are: (i) morphometric alterations in the mitochondria and melanosomes of melanoma cells treated with azelaic acid; (ii) deregulation of mitochondrial control and extramitochondrial metabolism in similarly treated cells; (iii) considerable acceleration of NAD(P) transient kinetics in atractylate-treated L sarcoma cells; (iv) alterations of mitochondria and Golgi in fusion-deficient myoblasts; (v) tentative recognition of beta-glucosidase deficiency in Gaucher disease cells by the use of fluorescent and fluorogenic lysosomal probes; and (vi) UVA-induced accumulation of Schiff bases (a kind of accelerated photo-aging) in yeast and kidney epithelial cells. Because these studies utilize probing at whatever points along the concerned pathways become accessible, at first glance they may look disconnected. What and where is the connecting thread, for instance, between studying melanoma metabolism, melanosome morphometry, hepatocyte organelle morphogenesis and transformation, myotube organelle morphogenesis and fusion-non-fusion, and lysosomal activity in gene-deficient cells? In the mapping of the regulatory and deregulatory mechanisms involved in the switching of differentiation or transformation, each of the above topics carries an information content towards resolution of the pleiotropic puzzle. The integration of such information with increasing resolution and access to intracellular microdomains may ultimately allow focus on the precise target, the switch from differentiation to transformation or vice versa.

Kohn MC, Melnick RL. **A physiological model for ligand-induced accumulation of alpha 2u globulin in male rat kidney: roles of protein synthesis and lysosomal degradation in the renal dosimetry of 2,4,4-trimethyl-2-pentanol.** Toxicology 1999;136(2-3):89-105.
A physiologically based pharmacokinetic (PBPK) model was constructed for the disposition of 2,4,4-

trimethyl-2-pentanol (TMP-2-OH) in male rats and its induction of accumulation of renal alpha2u-globulin (alpha2u). The model included diffusion-restricted delivery of TMP-2-OH to compartments representing liver, lung, fat, kidney, GI tract, aggregated rapidly perfused tissues, and aggregated slowly perfused tissues. Metabolism by oxidation and glucuronidation was included for liver and kidneys. Rates of hepatic alpha2u production and resorption by renal proximal tubules were taken from the literature. Degradation of liganded alpha2u by renal lysosomal cathepsins was modeled with a Km value corresponding to the measured 30% reduction in proteolytic efficiency and with free and bound forms of alpha2u competing for access to the enzymes. Increased pinocytotic uptake of alpha2u into the kidney induces cathepsin activity. A model that ascribed renal alpha2u accumulation solely to reduced lysosomal proteolysis failed to reproduce the observed accumulation. The model could reproduce experimental observations if a transient increase in hepatic synthesis of alpha2u, stimulated by the presence of liganded alpha2u in the blood, and accelerated secretion of the protein from the liver were assumed. This model reproduces time course data of blood and kidney TMP-2-OH and renal alpha2u concentrations, suggesting that renal accumulation of alpha2u is not simply a consequence of reduced proteolytic degradation but may also involve a transient increase in hepatic alpha2u production. The model predicts increased delivery of TMP-2-OH to the kidney and consequent increased renal production of potentially toxic TMP-2-OH metabolites than would be the case if no alpha2u were present. Induced lysosomal activity and increased production of toxic metabolites may both contribute to the nephrotoxicity observed in male rats exposed to an alpha2u ligand or its precursor.

Lee D, Sohn H, Kalpana GV, Choe J. **Interaction of E1 and hSNF5 proteins stimulates replication of human papillomavirus DNA.** Nature 1999;399(6735):487-91.
Mammalian viruses often use components of the host's cellular DNA replication machinery to carry out replication of their genomes, which enables these viruses to be used as tools for characterizing factors that are involved in cellular DNA replication. The human papillomavirus (HPV) E1 protein is essential for replication of the virus DNA. Here we identify the cellular factor that participates in viral DNA replication by using a two-hybrid assay in the yeast Saccharomyces cerevisiae and E1 protein as bait. Using this assay, we isolated Inil/hSNF5, a component of the SWI/SNF complex which facilitates transcription by altering the structure of chromatin. In vitro binding and immunoprecipitation confirmed that E1 interacts directly with Ini1/hSNF5. Transient DNA-replication assay revealed that HPV DNA replication is stimulated in a dose-dependent manner by addition of Ini1/hSNF5, and that Ini1/hSNF5 antisense RNA blocks the replication of HPV DNA. Amino-acid substitution at residues that are conserved among E1 proteins prevented the E1-Ini1/hSNF5 interaction and reduced DNA replication of HPV in vivo. Our results indicate that Ini1/hSNF5 is required for the efficient replication of papillomavirus DNA and is therefore needed, either alone or in complex with SWI/SNF complex, for mammalian DNA replication as well.

Leng G, Lewalter J, Rohrig B, Idel H. **The influence of individual susceptibility in pyrethroid exposure.** Toxicol Lett 1999;107(1-3):123-30.
The aim of this study was to find a suitable biomarker for pyrethroid adverse effects. It was shown that there is a correlation between the half-life time (t(1/2)) of pyrethroids in plasma and the clinical findings. We hypothized that this finding indicates an interindividual different amount of total esterase activity or even a polymorphism. By in vitro experiments it was demonstrated that pyrethroids are cleaved by

carboxylesterases. After it turned out that carboxylesterase activity in human plasma is too low for detection, a method for specific determination of carboxylesterase activity in human isolated lymphocytes was developed. As a substrate for carboxylesterase activity, cyfluthrin was added to the lymphocyte suspension. As a proof for cyfluthrin degradation by carboxylesterases the produced hydrocyanic acid was determined by GC/MS. First hints for interindividual differences in carboxylesterase activity in lymphocytes were found.

Lentz DL, Clark AM, Hufford CD, Meurer-Grimes B, Passreiter CM, Cordero J, Ibrahimi O, Okunade AL. **Antimicrobial properties of Honduran medicinal plants.** J Ethnopharmacol 1998;63(3):253-63.
Ninety-two plants used in the traditional pharmacopoeia of the Pech and neighboring Mestizo peoples of central Honduras are reported. The results of in vitro antimicrobial screens showed that 19 of the extracts from medicinal plants revealed signs of antifungal activity while 22 demonstrated a measurable inhibitory effect on one or more bacterial cultures. Bioassay-guided fractionation of extracts from Mikania micrantha, Neurolaena lobata and Piper aduncum produced weak to moderately active isolates. The broad spectrum of activity of the extracts helps to explain the widespread use of these plants for wound healing and other applications.

Li AP, Lu C, Brent JA, Pham C, Fackett A, Ruegg CE, Silber PM. **Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential.** Chem Biol Interact 1999;121(1):17-35.
Cryopreserved human hepatocytes were extensively characterized in our laboratory. The post-thaw viability, measured via dye exclusion, ranged from 55 to 83%, for hepatocytes cryopreserved from 17 donors. Post-thaw viability and yield (viable cells per vial) were found to be stable up to the longest storage duration evaluated of 120 days. Drug-metabolizing enzyme activities of the cryopreserved hepatocytes (mean of ten donors) as percentages of the freshly isolated cells were: 97%, for cytochrome P450 isoform (CYP) 1A2, 78% for CYP2A6, 96% for CYP2C9. 86% for CYP2Cl9, 90% for CYP2D6, 164% for CYP3A4, 76% for UDP-glucuronidase, and 88% for umbelliferone sulfotransferase. Known species-differences in 7-ethoxycoumarin (7-EC) metabolism were reproduced by cryopreserved hepatocytes from human, rat, rabbit, dog, and monkey, illustrating the utility of cryopreserved hepatocytes from multiple animal species in the evaluation of species-differences in drug metabolism. Higher throughput screening (HTS) assays were developed using cryopreserved human hepatocytes for hepatotoxicity, metabolic stability, and inhibitory drug-drug interactions. Dose-dependent cytotoxicity, measured using MTT metabolism as an endpoint, was observed for the known hepatotoxic chemicals tamoxifen, clozapine, cadmium chloride, diclofenac, amiodarone, tranylcypromine, precocene II, but not for 2-thiouracil. Cell density- and time-dependent metabolism of 7-EC and dextromethorphan were observed in the HTS assay for metabolic stability. Known CYP isoform-specific inhibitors were evaluated in the HTS assay for inhibitory drug-drug interactions. Furafylline, sulfaphenazole, quinidine, and ketoconazole were found to be specific inhibitors of CYP1A2, CYP2C9, CYP2D6, and CYP3A4, respectively. Tranylcypromine and diethyldithiocarbamate were found to be less specific, with inhibitory effects towards several CYP isoforms, including CYP2A6, CYP2C9, CYP2C19, and CYP2E1. These results suggest that cryopreserved human hepatocytes represent a useful experimental tool for the evaluation of drug metabolism, toxicity, and inhibitory drug-drug interaction potential.

Li TW, Wang J, Lam JT, Gutierrez EM, Solorzano-Vargus RS, Tsai HV, Martin MG. **Transcriptional control of the murine polymeric IgA receptor promoter by glucocorticoids.** Am J Physiol 1999;276 (6 Pt 1):1425-34.
Glucocorticoids have been implicated as an important regulator of intestinal epithelial cell ontogeny. The polymeric IgA receptor (pIgR) is expressed in the intestinal epithelial layer and is regulated by several mediators, including glucocorticoids. The mechanism of how corticosteroids alter the transcriptional regulation of pIgR expression has not been defined. In this study, we demonstrated that glucocorticoids upregulate steady-state pIgR mRNA levels in the proximal intestine of suckling rats and in the IEC-6 intestinal cell line. We performed functional analysis of the 5'-flanking region in the presence of glucocorticoids and its receptor using the intestinal cell line Caco-2. We screened 4.7 kb of the upstream region of the murine gene and identified the most potent steroid response element to reside between nt -215 and -163 relative to the start of transcription. Substitution mutation analysis of this region identified the location of the putative steroid response element to be between nt -195 and -176. In vitro DNase I footprint analysis using the recombinant glucocorticoid receptor DNA binding domain confirmed a single area of protection that spans the nt identified by mutagenesis analysis. Electrophoretic mobility shift assays of the putative element confirmed the binding of both recombinant and cell synthesized glucocorticoid receptor in a specific manner. In summary, we report the identification and characterization of the glucocorticoid-DNA response element located in the immediate 5'-upstream region of the murine pIgR gene.

Lim Y, Kim JH, Kim KA, Chang HS, Park YM, Ahn BY, Phee YG. **Silica-induced apoptosis in vitro and in vivo.** Toxicol Lett 1999;108(2-3):335-9.
Silica exposure results in an initially acute inflammatory response followed by chronic fibrotic change. The mechanism for the maintenance of silica-induced inflammation has not been understood yet. In silica-induced acute inflammation and chronic fibrosis, various mediators such as reactive oxygen species, cytokines and growth factors are released. And these substances are suggested to have the regulatory role for the inflammation and fibrosis by possessing the potential to influence apoptosis. To demonstrate the apoptosis as an underlying mechanism for the development of silicosis, in vitro and in vivo models were designed. In in vitro study, we evaluated that apoptotic cell fraction in silica (10, 50 microg/cm2)-treated A549 cells was significantly increased in comparison with control by FACS (fluorescein activated cell sorter). Also genomic DNA from silica (10, 50 microg/cm2)-treated A549 showed DNA ladder formation while control and 1 microg/cm2 groups didn't. In in vivo study, total cell numbers and apoptotic cell numbers of BAL (bronchoalveolar lavage) fluid from silica (10, 20, 40 mg/kg)-instilled rats were significantly higher than control group from 1 week. From these results, we concluded acute and chronic presence of apoptosis may contributes to silica-induced acute inflammation and chronic fibrosis.

Lizard G, Monier S, Cordelet C, Gesquiere L, Deckert V, Gueldry S, Lagrost L, Gambert P. **Characterization and comparison of the mode of cell death, apoptosis versus necrosis, induced by 7beta-hydroxycholesterol and 7-ketocholesterol in the cells of the vascular wall.** Arterioscler Thromb Vasc Biol 1999;19(5):1190-200.
Oxidized low density lipoproteins (LDLs) play a central role in atherosclerosis, and their toxicity is due, at least in part, to the formation of oxysterols that have been shown to induce apoptosis in various cell

types. As 7beta-hydroxycholesterol and 7-ketocholesterol are the major oxysterols found in oxidized LDLs, we have investigated and compared the mode of cell death, apoptosis versus necrosis, that they induce in the cells of the vascular wall, ie, endothelial cells, smooth muscle cells, and fibroblasts. To this end, human vascular endothelial cells from umbilical cord veins (HUVECs), human artery smooth muscle cells, A7R5 rat smooth muscle cells, MRC5 human fibroblasts, and human fibroblasts isolated from umbilical cord veins were taken at confluence and incubated for 48 hours with 7beta-hydroxycholesterol or 7-ketocholesterol (concentration range, 5 to 80 microg/mL). In all cells, both 7beta-hydroxycholesterol and 7-ketocholesterol exhibited toxic effects characterized by a loss of cell adhesion and an increased permeability to propidium iodide. In oxysterol-treated endothelial and smooth muscle cells, typical features of apoptosis were revealed: condensed and/or fragmented nuclei were detected by fluorescence microscopy after staining with Hoechst 33342, oligonucleosomal DNA fragments were visualized in situ in the cell nuclei by the TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method, and internucleosomal DNA fragmentation was found on agarose gel. In contrast, in oxysterol-treated fibroblasts, fragmented and/or condensed nuclei were never revealed, and no DNA fragmentation was observed either by the TUNEL method or by DNA analysis on agarose gel, indicating that these oxysterols induced necrosis in these cells but not apoptosis. In addition, acetylated Asp-Glu-Val-L-aspartic acid aldehyde (an inhibitor of Asp-Glu-Val-L-aspartic acid-sensitive caspases) prevented 7beta-hydroxycholesterol- and 7-ketocholesterol-induced cell death in HUVECs and smooth muscle cells but not in fibroblasts. Thus, 7beta-hydroxycholesterol and 7-ketocholesterol have dual cytotoxic effects on the cells of the vascular wall by their ability to induce apoptosis in endothelial and smooth muscle cells and necrosis in fibroblasts.

Lo KW, Zhang Q, Li M, Zhang M. **Apoptosis-linked gene product ALG-2 is a new member of the calpain small subunit subfamily of Ca2+-binding proteins.** Biochemistry 1999;38(23):7498-508. ALG-2 is a newly discovered Ca2+-binding protein which has been demonstrated to be directly linked to apoptosis. Structurally, ALG-2 is expressed as a single polypeptide chain corresponding to a 22 kDa protein containing five putative EF-hand Ca2+-binding sites. In this work, we have developed an efficient expression and purification scheme for recombinant ALG-2. Utilizing this protocol, we can routinely obtain purified recombinant protein with a yield of approximately 100 mg per liter of bacterial cell cultures. Gel filtration and chemical cross-linking experiments have shown that Ca2+-free ALG-2 forms a weak homodimer in solution. Biochemical and spectroscopic studies of truncated and point mutants of ALG-2 demonstrated that the fifth EF-hand Ca2+-binding motif is likely to participate in the formation of the dimer complex. Experimentally, both the amino- and carboxyl-terminal truncated mutants of ALG-2 have shown their ability to retain the structural, as well as, Ca2+-binding integrity when individually expressed in bacteria. In this respect, the N-terminal domain encompasses the first two EF-hands, and the C-terminal domain contains the remaining three EF-hands. Combining mutagenesis and spectroscopic studies, we showed that ALG-2 possesses two strong Ca2+-binding sites. Employing fluorescence spectroscopy and circular dichroism, we showed that the binding of Ca2+ to ALG-2 induced significant conformational changes in both the N-terminal and C-terminal domains of the protein. Furthermore, our studies demonstrated that Ca2+ binding to both strong Ca2+-binding sites of ALG-2 is required for ion-induced aggregation of the protein. We also report here the expression, purification, and partial characterization of a Ca2+-binding-deficient ALG-2 mutant (Glu47Ala/Glu114Ala). In light of its much decreased affinity for Ca2+, this mutant could prove to be instrumental

in elucidating the Ca2+-mediated function of ALG-2 within the context of its cellular environment.

Lowe GM, Booth LA, Young AJ, Bilton RF. **Lycopene and beta-carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses.** Free Radic Res 1999;30(2):141-51.
Epidemiological studies have clearly demonstrated a link between dietary carotenoids and the reduced incidence of certain diseases, including some cancers. However recent intervention studies (e.g. ATBC, CARET and others) have shown that beta-carotene supplementation has little or no beneficial effect and may, in fact, increase the incidence of lung cancers in smokers. This presents a serious dilemma for the scientific community - are carotenoids at high concentrations actually harmful in certain circumstances? Currently, a significant number of intervention studies are on-going throughout the world involving carotenoids (of both natural and synthetic origin). Our approach has been to study the ability of supplementary carotenoids in protecting cells against oxidatively-induced DNA damage (as measured by the comet assay), and membrane integrity (as measured by ethidium bromide uptake). Both lycopene and beta-carotene only afforded protection against DNA damage (induced by xanthine/xanthine oxidase) at relatively low concentrations (1-3 microM). These levels are comparable with those seen in the plasma of individuals who consume a carotenoid-rich diet. However, at higher concentrations (4-10 microM), the ability to protect the cell against such oxidative damage was rapidly lost and, indeed, the presence of carotenoids may actually serve to increase the extent of DNA damage. Similar data were obtained when protection against membrane damage was studied. This would suggest that supplementation with individual carotenoids to significantly elevate blood and tissue levels is of little benefit and, may, in fact, be deleterious. This in vitro data presented maybe significant in the light of recent intervention trials.

Mcguinness SM, Johansson R, Lundstrom J, Ross D. **Induction of apoptosis by remoxipride metabolites in HL60 and CD34+/CD19- human bone marrow progenitor cells: potential relevance to remoxipride-induced aplastic anemia.** Chem Biol Interact 1999;121(3):253-65.
The antipsychotic agent, remoxipride [(S)-(-)-3-bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxy- benz amide] has been associated with acquired aplastic anemia. We have examined the ability of remoxipride, three pyrrolidine ring metabolites and five aromatic ring metabolites of the parent compound to induce apoptosis in HL60 cells and human bone marrow progenitor (HBMP) cells. Cells were treated for 0-24 h with each compound (0-200 microM). Apoptosis was assessed by fluorescence microscopy in Hoechst 33342- and propidium iodide stained cell samples. Results were confirmed by determination of internucleosomal DNA fragmentation using gel electrophoresis for HL60 cell samples and terminal deoxynucleotidyl transferase assay in HBMP cells. The catechol and hydroquinone metabolites, NCQ436 and NCQ344, induced apoptosis in HL60 and HBMP cells in a time- and concentration dependent manner, while the phenols, NCR181, FLA873, and FLA797, and the derivatives formed by oxidation of the pyrrolidine ring, FLA838, NCM001, and NCL118, had no effect. No necrosis was observed in cells treated with NCQ436 but NCQ344 had a biphasic effect in both cell types, inducing apoptosis at lower concentrations and necrosis at higher concentrations. These data show that the catechol and hydroquinone metabolites of remoxipride have direct toxic effects in HL60 and HBMP cells, leading to apoptosis, while the phenol metabolites were inactive. Similarly, benzene-derived catechol and hydroquinone, but not phenol, induce apoptosis in HBMP cells [Moran et al., Mol.

Pharmacol., 50 (1996) 610-615]. We propose that remoxipride and benzene may induce aplastic anemia via production of similar reactive metabolites and that the ability of NCQ436 and NCQ344 to induce apoptosis in HBMP cells may contribute to the mechanism underlying acquired aplastic anemia that has been associated with remoxipride.

Migheli R, Godani C, Sciola L, Delogu MR, Serra PA, Zangani D, De Natale G, Miele E, Desole MS. **Enhancing effect of manganese on L-DOPA-induced apoptosis in PC12 cells: role of oxidative stress.** J Neurochem 1999;73(3):1155-63.
L-DOPA and manganese both induce oxidative stress-mediated apoptosis in catecholaminergic PC12 cells. In this study, exposure of PC12 cells to 0.2 mM MnCl2 or 10-20 microM L-DOPA neither affected cell viability, determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, nor induced apoptosis, tested by flow cytometry, fluorescence microscopy, and the TUNEL technique. L-DOPA (50 microM) induced decreases in both cell viability and apoptosis. When 0.2 mM MnCl2 was associated with 10, 20, or 50 microM L-DOPA, a concentration-dependent decrease in cell viability was observed. Apoptotic cell death also occurred. In addition, manganese inhibited L-DOPA effects on dopamine (DA) metabolism (i.e., increases in DA and its acidic metabolite levels in both cell lysate and incubation medium). The antioxidant N-acetyl-L-cysteine significantly inhibited decreases in cell viability, apoptosis, and changes in DA metabolism induced by the manganese association with L-DOPA. An increase in autoxidation of L-DOPA and of newly formed DA is suggested as a mechanism of manganese action. These data show that agents that induce oxidative stress-mediated apoptosis in catecholaminergic cells may act synergistically.

Miller CL, Eaves CJ. **Expansion in vitro of adult murine hematopoietic stem cells with transplantable lympho-myeloid reconstituting ability.** Proc Natl Acad Sci U S A 1997 Dec 9;94 (25):13648-53.
Elucidation of mechanisms that regulate hematopoietic stem cell self-renewal and differentiation would be facilitated by the identification of defined culture conditions that allow these cells to be amplified. We now demonstrate a significant net increase (3-fold, $P < 0.001$) in vitro of cells that are individually able to permanently and competitively reconstitute the lymphoid and myeloid systems of syngeneic recipient mice when Sca-1(+)lin- adult marrow cells are incubated for 10 days in serum-free medium with interleukin 11, flt3-ligand, and Steel factor. Moreover, the culture-derived repopulating cells continued to expand their numbers in the primary hosts at the same rate seen in recipients of noncultured stem cells. In the expansion cultures, long-term culture-initiating cells increased 7- +/- 2-fold, myeloid colony-forming cells increased 140- +/- 36-fold, and total nucleated cells increased 230- +/- 62-fold. Twenty-seven of 100 cultures initiated with 15 Sca-1(+)lin- marrow cells were found to contain transplantable stem cells 10 days later. This frequency of positive cultures is the same as the frequency of transplantable stem cells in the original input suspension, suggesting that most had undergone at least one self-renewal division in vitro. No expansion of stem cells was seen when Sca-1+TER119- CD34+ day 14.5 fetal liver cells were cultured under the same conditions. These findings set the stage for further investigations of the mechanisms by which cytokine stimulation may elicit different outcomes in mitotically activated hematopoietic stem cells during ontogeny and in the adult.

Miyoshi H, Rust C, Roberts PJ, Burgart LJ, Gores GJ. **Hepatocyte apoptosis after bile duct ligation in the mouse involves Fas [see comments]**. Gastroenterology 1999;117(3):669-77.

BACKGROUNS & AIMS: Cholestatic liver injury results from the intrahepatic accumulation of toxic bile salts. Toxic bile salt-induced hepatocyte apoptosis in vitro is Fas dependent. The aim of this study was to ascertain if hepatocyte apoptosis in vivo during cholestasis is Fas dependent. METHODS: Studies were performed in bile duct-ligated (BDL) Fas-deficient lpr (lymphoproliferation) and wild-type mice. RESULTS: Hepatocyte apoptosis was the predominant mechanism of cell death as determined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling and trypan blue assays to quantitate apoptosis and necrosis. The mechanisms of hepatocyte apoptosis were dependent on the presence or absence of the Fas receptor and the duration of BDL. After BDL of 3 days' duration, increased hepatocyte apoptosis occurred only in wild-type but not lpr mice, indicating the apoptosis was Fas dependent. In contrast, after BDL of >/=7 days, hepatocyte apoptosis also occurred in lpr animals consistent with a Fas-independent mechanism of apoptosis. Hepatocyte apoptosis in BDL lpr mice was associated with an increase in Bax expression and Bax association with mitochondria. CONCLUSIONS: During extrahepatic cholestasis, hepatocyte apoptosis is mediated by Fas. However, in the absence of the Fas receptor, additional mechanisms of hepatocyte apoptosis occur. Inhibition of multiple apoptotic pathways is necessary to attenuate chronic cholestatic liver injury.

Moll TS, Elfarra AA. **Characterization of the reactivity, regioselectivity, and stereoselectivity of the reactions of butadiene monoxide with valinamide and the N-terminal valine of mouse and rat hemoglobin.** Chem Res Toxicol 1999;12(8):679-89.
Occupational exposure to 1,3-butadiene (BD) has been monitored by measuring the level of hemoglobin N-terminal valine adduct formation with the primary reactive metabolite, butadiene monoxide (BMO). However, mechanistic details concerning the relative reactivity, regioselectivity, and stereospecificity of BMO with the N-terminal valine of hemoglobin are lacking. In the studies presented here, L-valinamide was used as a model for the N-terminal valine of hemoglobin to compare the nucleophilic reactivity, regioselectivity, and stereoselectivity of the reaction both in aqueous solution and within a protein microenvironment. Four products produced by the reaction of L-valinamide with racemic BMO (two pairs of diastereomers produced by reactions at C-1 and C-2 of the epoxide moiety) were synthesized, purified, and characterized by (1)H NMR and GC/MS. These four reaction products were used as analytical standards for kinetic studies of the reaction of valinamide with BMO at physiological pH (7.4) and temperature (37 degrees C). The results show that the adducts formed by reaction at C-2 were formed at a ratio of approximately 2:1 compared to the adducts formed by reaction at C-1. The stereoisomers of each respective regioisomer were produced with similar rates of formation. The reaction of BMO with the N-terminal valine of hemoglobin was also studied in vitro using intact erythrocytes from Sprague-Dawley rats and B6C3F1 mice. After cleavage of the N-modified valine by the N-alkyl Edman degradation procedure using pentafluorophenylisothiocyanate (PFPITC), a novel procedure was developed that allowed GC/MS detection and quantitation of the four expected products by silylation of the PFPTH-valine-BMO derivatives. The hemoglobin results contrast with the valinamide results in that the reaction of BMO with the N-terminal valine residue in both rat and mouse hemoglobin produced mostly C-1 adducts. The rates obtained with rat hemoglobin were much slower than the rates obtained with mouse hemoglobin or with valinamide. These results, and the finding that the reaction with rat hemoglobin produced a higher ratio of C1:C2 adducts in comparison with the reaction with mouse hemoglobin, indicate the importance of measuring all four adducts when comparing the relative rates of adduct formation both with model compounds and among different species.

Monroy-Noyola A, Sogorb MA, Vilanova E. **Enzyme concentration as an important factor in the in vitro testing of the stereospecificity of the enzymatic hydrolysis of organophosphorus compounds.** Toxicol In Vitro 1999;13(4-5):689-92.
BIOSIS COPYRIGHT: BIOL ABS. A report is made of important differences in the Ca2+-dependent hydrolysis of the chiral phosphoramidate O-hexyl O-2,5-dichlorophenyl phosphoramidate (HDCP) when recorded using different quantities of hen liver microsomes. In a colorimetric microassay using the microsomes from 5 mg tissue in the presence of HDCP stereoisomers and 2.5 mM calcium, the R-HDCP isomer was hydrolysed at a rate similar to or slightly faster than S-HDCP isomer (14% v. 11%), while the S-HDCP stereoisomer was hydrolysed f amount of liver microsomes (range one- to threefold). This study demonstrates that the concentration of the subcellular fraction in in vitro assays is a critical factor to be taken into account in securing a more realistic approximation to the stereospecific enzymatic processes occurring in biological systems. Our data concerning the hydrolysis of HDCP by liver microsomes at high enzyme concentrations afford a better fit to the in vivo toxicological response with HDCP than assays performed with.

Newman WH, Zunzunegui RG, Warejcka DJ, Dalton ML, Castresana MR. **A reactive oxygen-generating system activates nuclear factor-kappaB and releases tumor necrosis factor-alpha in coronary smooth muscle cells.** J Surg Res 1999;85(1):142-7.
BACKGROUND: Recently we reported that bacterial lipopolysaccharide (LPS) stimulates release of tumor necrosis factor alpha (TNF-alpha) from porcine coronary arteries and smooth muscle cells cultured from those vessels. It has also been reported that plasma levels of TNF-alpha are elevated after myocardial infarction. Since it is known that the production of reactive oxygen intermediates (ROI) occurs during ischemia and ROI are suggested activators of the nuclear regulatory factor kappaB (NF-kappaB), we tested the hypothesis that release of TNF-alpha from smooth muscle cells could also be stimulated with a ROI-generating system. MATERIALS AND METHODS: Smooth muscle cells were isolated from porcine coronary arteries. Confluent cells in 48-well culture dishes were treated for 30 min with 0.003 units/ml xanthine oxidase (XO) and 2 mM hypoxanthine (HX) added to the culture medium. The medium was then removed and the cells were washed three times and fresh medium without HX-XO was added. Then, at 1, 3, and 6 h the medium was removed and analyzed for biologically active TNF-alpha. In other experiments, smooth muscle cells were treated with 20 micrograms/ml LPS for 6 h and aliquots of medium analyzed for TNF-alpha. Untreated cells served as controls. Data were analyzed by two-way ANOVA with repeated measures. Extracts of total cell protein were prepared and activation of NF-kappaB was determined by electrophoretic mobility shift assay. RESULTS: Treatment of cells with HX-XO stimulated release of TNF-alpha, which rose to a maximum of 17.5 +/- 1.7 units/mg cell protein at 6 h. This was significantly higher ($P < 0.05$) than release stimulated by LPS (10.2 +/- 1.0 units/mg at 6 h) or TNF-alpha detected in the culture medium from untreated control cells (4.2 +/- 0.9 units/mg protein at 6 h). Both HX/XO and LPS activated NF-kappaB. CONCLUSIONS: These results support the conclusion that coronary smooth muscle cells are a potential source of TNF-alpha during events that are associated with formation of ROI such as myocardial ischemia. Copyright 1999 Academic Press.

Nikolic D, Fan PW, Bolton JL, Van Breemen RB. **Screening for xenobiotic electrophilic metabolites using pulsed ultrafiltration-mass spectrometry.** Comb Chem High Throughput Screen 1999;2(3):165-

75.
A pulsed ultrafiltration-mass spectrometric screening assay has been developed to generate and identify electrophilic metabolites of xenobiotic compounds formed by hepatic cytochrome P450 enzymes. This assay would be suitable for the early identification of potentially toxic compounds during the initial phase of drug development. Rat liver microsomes were trapped by an ultrafiltration membrane in a stirred flow-through chamber, and substrates for microsomal cytochrome P450 including hydroxychavicol, 3-methylindole, cyproheptadine and 2-tert-butyl-4,6-dimethylphenol were flow-injected individually through the chamber along with the cofactors, NADPH and glutathione. Metabolites and glutathione conjugates were detected on-line using electrospray mass spectrometry. Alternatively, the ultrafiltrate was concentrated on a reversed phase HPLC column and analyzed using electrospray LC-MS or LC-MS-MS to separate and characterize isomeric metabolites and metabolites present at low concentration. Enzymatic activation of each xenobiotic substrate produced highly electrophilic metabolites such as quinones, quinone methides and imine methides that reacted with glutathione on-line to produce glutathione conjugates which were detected by using electrospray mass spectrometry. Although epoxides such as cyproheptadine epoxide were generated, it is likely that these compounds were insufficiently reactive to form glutathione conjugates in the absence of cytosolic glutathione S-transferases. Pulsed ultrafiltration-electrospray mass spectrometry offers an efficient method for in vitro formation and mass spectrometric characterization of activated microsomal drug metabolites and is suitable for use during the drug discovery process for the early identification and screening out of potentially toxic lead compounds.

Nishigaki R, Mitani H, Tsuchida N, Shima A. **Effect of cyclobutane pyrimidine dimers on apoptosis induced by different wavelengths of UV.** Photochem Photobiol 1999;70(2):228-35.
BIOSIS COPYRIGHT: BIOL ABS. Ultraviolet radiation within three different wavelength ranges, UVA (340-400 nm), UVB (290-320 nm) or UVC (200-290 nm), was shown to induce apoptosis in OCP13 cells, derived from the medaka fish. Morphological changes such as cell shrinkage and a decrease in the number of nucleoli appeared 4 h after UVA, UVB or UVC irradiation, although with different relative efficiencies. Doses required to induce apoptosis with similar efficiencies were about 2500-fold higher for UVA and 10-fold higher for UVB ed by photorepair treatment. (4) Morphological changes did not occur in cells attached to glass coverslips but only those in plastic dishes. (5) Apoptosis occurred without detectable increase of caspase-3-like activity. (6) Morphological changes were inhibited by N-acetylcysteine, a scavenger of active oxygen species. These results suggest the existence of two different pathways leading to apoptosis, one for long- (UVA) and the other for short- (UVB or UVC) wavelength radiation.

Noort D, Hulst AG, De Jong LP, Benschop HP. **Alkylation of human serum albumin by sulfur mustard in vitro and in vivo: mass spectrometric analysis of a cysteine adduct as a sensitive biomarker of exposure.** Chem Res Toxicol 1999;12(8):715-21.
To develop a mass spectrometric assay for the detection of sulfur mustard adducts with human serum albumin, the following steps were performed: quantitation of the binding of the agent to the protein by using [(14)C]sulfur mustard and analysis of acidic and tryptic digests of albumin from blood after exposure to sulfur mustard for identification of alkylation sites in the protein. The T5 fragment containing an alkylated cysteine could be detected in the tryptic digest with micro-LC/tandem MS

analysis. Attempts to decrease the detection limit for in vitro exposure of human blood by analysis of the alkylated T5 fragment were not successful. After Pronase treatment of albumin, S-[2-[(hydroxyethyl) thio]ethyl]Cys-Pro-Phe was analyzed by means of micro-LC/tandem MS, allowing a detection limit for in vitro exposure of human blood of 10 nM, which is 1 order of magnitude lower than that obtained by means of modified Edman degradation. The analytical procedure could be successfully applied to the analysis of albumin samples from Iranian victims of the Iran-Iraq war.

Nylander S, Kalies I. **Brefeldin A, but not monensin, completely blocks CD69 expression on mouse lymphocytes: efficacy of inhibitors of protein secretion in protocols for intracellular cytokine staining by flow cytometry.** J Immunol Methods 1999;224(1-2):69-76.
Flow cytometry is increasingly used for cytokine detection where it serves to complement ELISA (enzyme-linked immunosorbent assay) and ELISPOT assays. Since it is possible to stain both extracellular epitopes and intracellular cytokines on the same cells, this is a powerful technique for analysing cytokine expression in defined cell populations. However unstimulated cells do not express cytokines. Thus, appropriate stimulation is a prerequisite for studying cytokine expression. Here phorbol 12-myristate 13-acetate (PMA)/ionomycin in vitro stimulation has been applied. In order to accumulate the cytokines within the cells, protein secretion needs to be inhibited, by the addition of reagents that inhibit protein secretion during the stimulation. The two most widely used reagents are monensin and brefeldin A (BFA). These reagents differ somewhat in their mode of action, which might explain their different effects. Monensin is an inhibitor of trans-Golgi function, while BFA inhibits protein transport between the endoplasmic reticulum (ER) and the Golgi. CD69, a very early activation marker on lymphocytes and neutrophils, was monitored in order to measure the efficacy of the protein secretion inhibition. Here we report that: (a) BFA, but not Monensin, is able to completely block extracellular CD69 expression on mice splenocytes after in vitro stimulation with PMA/ionomycin; (b) Monensin is more toxic than BFA and increases the relative amount of CD4+ cells due to a more profound increase in dead cells in the CD4- population; (c) CD69 is a useful marker when setting up intracellular staining of cytokines for flow cytometry.

O'Brien KA, Lemke SJ, Cocke KS, Rao RN, Beckmann RP. **Casein kinase 2 binds to and phosphorylates BRCA1.** Biochem Biophys Res Commun 1999;260(3):658-64.
The BRCA1 gene encodes a complex protein that appears to be involved in some aspects of DNA repair, transcription, or cell cycle regulation. The phosphorylation of BRCA1 is enhanced following episodes of DNA damage or during cell cycle progression, indicating that phosphorylation may be an important regulatory mechanism. Through a yeast two hybrid assay, we found that the beta-subunit of casein kinase 2 (CK2) associated with a carboxy-terminal region of BRCA1. This association was much weaker with the same fragment bearing a missense mutation (M1775R) that has been identified in breast tumors. The interaction was also evident in Sf9 cells. Subsequent studies showed that BRCA1 was phosphorylated in vitro by CK2. An analysis by site directed mutagenesis of BRCA1 showed that in vitro phosphorylation by CK2 required a serine at aa1572. These data implicate CK2 as a potential mediator of BRCA1 activity. Copyright 1999 Academic Press.

Okada K, Wangpoengtrakul C, Osawa T, Toyokuni S, Tanaka K, Uchida K. **4-Hydroxy-2-nonenal-mediated impairment of intracellular proteolysis during oxidative stress. Identification of proteasomes as target molecules.** J Biol Chem 1999;274(34):23787-93.

Oxidative stress is associated with important pathophysiological events in a variety of diseases. It has been postulated that free radicals and lipid peroxidation products generated during the process may be responsible for these effects because of their ability to damage cellular components such as membranes, proteins, and DNA. In the present study, we provide evidence that oxidative stress causes a transient impairment of intracellular proteolysis via covalent binding of 4-hydroxy-2-nonenal (HNE), a major end product of lipid peroxidation, to proteasomes. A single intraperitoneal treatment with the renal carcinogen, ferric nitrilotriacetate, caused oxidative stress, as monitored by accumulation of lipid peroxidation products and 8-hydroxy-2'-deoxyguanosine, in the kidney of mice. In addition, transient accumulation of HNE-modified proteins in the kidney was also found by competitive enzyme-linked immunosorbent assay and immunohistochemical analyses. This and the observation that the HNE-modified proteins were significantly ubiquitinated suggested a crucial role of proteasomes in the metabolism of HNE-modified proteins. In vitro incubation of the kidney homogenates with HNE indeed resulted in a transient accumulation of HNE-modified proteins, whereas the proteasome inhibitor significantly suppressed the time-dependent elimination of HNE-modified proteins. We found that, among three proteolytic activities (trypsin, chymotrypsin, and peptidylglutamyl peptide hydrolase activities) of proteasomes, both trypsin and peptidylglutamyl peptide hydrolase activities in the kidney were transiently diminished in accordance with the accumulation of HNE-modified proteins during oxidative stress. The loss of proteasome activities was partially ascribed to the direct attachment of HNE to the protein, based on the detection of HNE-proteasome conjugates by an immunoprecipitation technique. These results suggest that HNE may contribute to the enhanced accumulation of oxidatively modified proteins via an impairment of ubiquitin/proteasome-dependent intracellular proteolysis.

Onderwater RC, Commandeur JN, Menge WM, Vermeulen NP. **Activation of microsomal glutathione S-transferase and inhibition of cytochrome P450 1A1 activity as a model system for detecting protein alkylation by thiourea-containing compounds in rat liver microsomes.** Chem Res Toxicol 1999;12(5):396-402.

The recent development of several promising new thiourea-containing drugs has renewed interest in the thiourea functionality as a potential toxicophore. Most adverse reactions of thiourea-containing compounds are attributed to the thionocarbonyl moiety. Oxidation of these thionocarbonyl compounds by flavin-containing monooxygenases (FMO) and cytochrome P450 isoenzymes (P450) to reactive sulfenic, sulfinic, or sulfonic acids leads to alkylation of essential macromolecules. To more rationally design thiourea-containing drugs, structure-toxicity relationships (STRs) must be derived. Since for the development of STRs a large number of thiourea-containing compounds must be investigated, it is important to develop rapid in vitro assays for alkylating potential. In this study, the utility of activation of microsomal glutathione S-transferase (mGST) and inactivation of P450 1A1 as markers of the alkylating potential of metabolites of thiourea-containing compounds was investigated. It was found that metabolites of thiourea-containing compounds inactivate P450 1A1 in a time-dependent manner, as evidenced by a decrease in 7-ethoxyresorufin O-dealkylation (EROD) activity. An extent of inactivation of P450 1A1 by 100 microM N-phenylthiourea (PTU) of 64% was found after 10 min. This inactivation was dependent on the presence of NADPH and the presence of the thionosulfur, since the carbonyl analogue of PTU was not found to inactivate P450 1A1, and was partially prevented by heat treatment of the microsomes which is known to selectively inactivate FMO enzymes. Inactivation of P450 1A1 could be reversed by treatment with dithiothreitol, indicating the formation of disulfide bonds. However,

thiourea-containing compounds also inhibited the EROD activity of P450 1A1 in a competitive manner. This property complicates the usefulness of the EROD activity of P450 1A1 as a marker for the alkylating potential of thiourea-containing compounds. It was found that metabolites of thiourea-containing compounds could transiently activate the mGST. A maximal level of activation by 100 microM PTU of 162+/-16% was found after 10 min. Activation of mGST by 100 microM PTU was dependent on the presence of NADPH and the presence of the thionosulfur, since the carbonyl analogue of PTU was not found to activate mGST. Activation was completely prevented by heat treatment of the microsomes, indicating involvement of FMO in the bioactivation process. Finally, a series of structurally diverse thiourea-containing compounds were tested for their ability to activate mGST. It appeared that their potency in alkylating mGST was inversely related to their Vmax/Km value for the FMO enzyme. From this study, it is concluded that, whereas activation of mGST in rat liver microsomes may be a useful system with which to investigate the relationship between structure and alkylating potential of thiourea-containing compounds in vitro, inactivation of P450 1A1 is not.

Ou YC, Thompson SA, Ponce RA, Schroeder J, Kavanagh TJ, Faustman EM. **Induction of the cell cycle regulatory gene p21 (Waf1, Cip1) following methylmercury exposure in vitro and in vivo.** Toxicol Appl Pharmacol 1999;157(3):203-12.
Methylmercury (MeHg) is recognized as a significant environmental hazard, particularly to the development of the nervous system. To study the molecular mechanisms underlying cell cycle inhibition by MeHg, we assessed the involvement of p21 (Waf1, Cip1), a cell cycle regulatory gene implicated in the G1 and G2 phases of cell cycle arrest, in primary embryonic cells and adult mice following MeHg exposure. Previous literature has supported the association of increased p21 expression with chondrocyte differentiation. In support of this finding, we observed an increasing p21 expression during limb bud (LB), but not midbrain central nervous system (CNS) cell differentiation. Both embryonic LB and CNS cells responded to MeHg exposure with a concentration-dependent increase in p21 mRNA. In the parallel adult study, C57BL/6 female mice were chronically exposed to 10 ppm MeHg via drinking water for 4 weeks. While there was limited or absent induction of Gadd45, Gadd153, and the gamma-glutamylcysteine synthetase catalytic subunit, p21 was markedly induced in the brain, kidney, and liver tissues in most of the animals that showed MeHg-induced behavioral toxicity such as hyperactivity and tremor. Furthermore, the induction of p21 mRNA was accompanied by an increase in p21 protein level. The results indicate that the activation of cell cycle regulatory genes may be one mechanism by which MeHg interferes with the cell cycle in adult and developing organisms. Continued examination of the molecular mechanisms underlying cell cycle inhibition may potentially lead to utilization of this mechanistic information to characterize the effects of MeHg exposure in vivo. Copyright 1999 Academic Press.

Pacchierotti F, Adler ID, Anderson D, Brinkworth M, Demopoulos NA, Lahdetie J, Osterman-Golkar S, Peltonen K, Russo A, Tates A, et al. **Genetic effects of 1,3-butadiene and associated risk for heritable damage.** Mutat Res 1998 Jan 16;397(1):93-115.
A summary of the results of the studies conducted in the EU Project "Multi-endpoint analysis of genetic damage induced by 1,3-butadiene and its major metabolites in somatic and germ cells of mice, rats and man" is presented. Results of the project are summarized on the detection of DNA and hemoglobin adducts, on the cytotoxic and clastogenic effects in somatic and germinal cells of mice and rats, on the

induction of somatic mutations at the hprt locus of experimental rodents and occupationally exposed workers, on the induction of dominant lethal mutations in mice and rats, and on heritable translocations induced in mice, after exposure to butadiene (BD) or its major metabolites, butadiene monoepoxide (BMO), diepoxybutane (DEB) and butadiene diolepoxide (BDE). The primary goal of this project was to collect experimental data on the genetic effects of BD in order to estimate the germ cell genetic risk to humans of exposure to BD. To achieve this, the butadiene exposure are based on data for heritable translocations and bone marrow micronuclei induced in mice and chromosome aberrations observed in lymphocytes of exposed workers. A doubling dose for heritable translocations in human germ cells of 4900 ppm/h is estimated, which, assuming cumulative BD exposure over the sensitive period of spermatogenesis, corresponds to 5-6 weeks of continuous exposure at the workplace to 20-25 ppm. Alternatively, the rate of heritable translocation induction per ppm/h of BD exposure is estimated to be approximately 0.8 per million live born, compared to a spontaneous incidence of balanced translocations in humans of approximately 800 per million live born. These estimates have large confidence intervals and are only intended to indicate orders of magnitude of human genetic risk. These risk estimates are based on data from germ cells of BD-exposed male mice. The demonstration that clastogenic damage was induced by DEB in preovulatory oocytes at doses which were not ovotoxic implies that additional studies on the response of mammalian female germ cells to BD and its metabolites are needed. The basic assumption of the above genetic risk estimates is that experimental mouse data obtained after BD exposure can be extrapolated to humans. Several points exist in the present report and in the literature which contradict this assumption: (1) the level of BMO-hemoglobin adducts was significantly elevated in BD-exposed workers; however, it was considerably lower than would have been predicted from comparable rat and mouse exposures; (2) the concentrations of the metabolites DEB and BMO were significantly higher in mouse than in rat blood after BD exposure. Thus, while metabolism of BD is qualitatively similar in the two species, it is quantitatively different; (3) no increase of HPRT mutations was shown in 19 workers exposed on average to 1.8 ppm of BD, while in a different population of workers from a US plant exposed on average to 3.5 ppm of BD, a significant increase of HPRT variants was detected; and (4) data from cancer bioassays and cancer epidemiology suggest that rat is a more appropriate model than mouse for human cancer risk from BD exposure. However, the dominant lethal study in rats gave a negative result. At present, we do not know which BD metabolite(s) may be responsible for the genetic effects even though the bifunctional alkylating agent DEB is the most likely candidate for the induction of clastogenic events. Unfortunately, methods to measure DEB adducts in hemoglobin or DNA are only presently being developed. Despite these several uncertainties the use of the mouse genetic data is regarded as a justifiable and conservative approach to human genetic risk estimation given the considerable heterogeneity observed in the biotransformation of BD in humans.

Paglia DE, Renner SW, Bhambhani K. **Differential effects of low-level lead exposure on the natural isozymes of erythrocyte 5'-nucleotidase.** Clin Biochem 1999;32(3):193-9.
OBJECTIVE: Erythrocyte pyrimidine 5'-nucleotidase (Pyr-5'-N) is highly sensitive to heavy metal inactivation in vitro and in vivo, and a number of studies have verified its usefulness as a biomarker of acute and chronic lead exposures. Retrospective and prospective studies attempted to determine whether the known linearity of Pyr-5'-N inhibition by lead concentrations above 40 microg/dL whole blood might continue into the lowest range of exposures now considered to be toxic in children (<10-20 microg/dL), thereby extending its value as a biomarker of lead exposure. DESIGN: Activities of Pyr-5'-

N and a lead-insensitive isozyme, deoxyribonucleotidase (d-5'-N), were compared to blood lead and free erythrocyte protoporphyrin (FEP) concentrations. RESULTS: Pyr-5'-N activities in erythrocytes from 70 children displayed an inverse linear correlation with whole blood lead of 1-35 microg/dL, whereas d-5'-N did not correlate. There was no apparent minimum threshold for Pyr-5'-N inhibition by lead. CONCLUSIONS: Linearity of Pyr-5'-N inhibition by lead extends throughout the range of clinical concern in pediatric cases. Pyr-5'-N/d-5'-N activity ratios may provide an even more sensitive, internally controlled biomarker of low-level lead overburden, since both isozymes vary comparably in activity as a function of reticulocytosis and mean red cell age.

Parsons CG, Danysz W, Bartmann A, Spielmanns P, Frankiewicz T, Hesselink M, Eilbacher B, Quack G. **Amino-alkyl-cyclohexanes are novel uncompetitive NMDA receptor antagonists with strong voltage-dependency and fast blocking kinetics: in vitro and in vivo characterization.** Neuropharmacology 1999;38(1):85-108.

The present study characterized the in vitro NMDA receptor antagonistic properties of novel amino-alkyl-cyclohexane derivatives and compared these effects with their ability to block excitotoxicity in vitro and MES-induced convulsions in vivo. The 36 amino-alkyl-cyclohexanes tested displaced [3H]-(+)-MK-801 binding to rat cortical membranes with K(i)s between 1.5 and 143 microM. Current responses of cultured hippocampal neurones to NMDA were antagonized by the same compounds with a wide range of potencies (IC50s of 1.3-245 microM, at -70 mV) in a use- and strongly voltage-dependent manner (delta 0.55-0.87). The offset kinetics of NMDA receptor blockade was correlated with equilibrium affinity (Corr Coeff. 0.87 $P < 0.0001$). As an example, MRZ 2/579 (1-amino-1,3,3,5,5-pentamethyl-cyclohexane HCl) had similar blocking kinetics to those previously reported for memantine (K(on) 10.67 +/- 0.09 x 10(4) M(-1) s(-1), K(off) 0.199 +/- 0.02 s(-1), K(d) = K(off)/K(on) = 1.87 microM c.f. IC50 of 1.29 microM). Most amino-alkyl-cyclohexanes were protective against glutamate toxicity in cultured cortical neurones (e.g. MRZ 2/579 IC50 2.16 +/- 0.03 microM). Potencies in the three in vitro assays showed a relatively strong cross correlation (all corr. coeffs. > 0.72, $P < 0.0001$). MRZ 2/579 was also effective in protecting hippocampal slices against 7 min. hypoxia/hypoglycaemia-induced reduction of fEPSP amplitude in CA1 with an EC50 of 7.01 +/- 0.24 microM. MRZ 2/579 showed no selectivity between NMDA receptor subtypes expressed in Xenopus oocytes but was somewhat more potent than in patch clamp experiments-IC50s of 0.49 +/- 0.11, 0.56 +/- 0.01 microM, 0.42 +/- 0.04 and 0.49 +/- 0.06 microM on NR1a/2A /2B, /2C and 2/D, respectively. In contrast, memantine and amantadine were both 3-fold more potent at NR1a/2C and NR1a/2D than NR1a/2A receptors. All Merz amino-alkyl-cyclohexane derivatives inhibited MES-induced convulsions in mice with ED50s ranging from 3.6 to 130 mg/kg i.p. The in vivo and in vitro potencies correlated indicating similar access of most compounds to the CNS. MRZ 2/579 administered at 10 mg/kg resulted in peak plasma concentrations of 5.3 and 1.4 microM following i.v. and p.o. administration respectively, which then declined with a half life of around 170-210 min. Analysis of A.U.C. concentrations indicates a p.o./i.v. bioavailability ratio for MRZ 2/579 of 60%. MRZ 2/579 injected i.p. at a dose of 5 mg/kg resulted in peak brain extracellular fluid (ECF) concentrations of 0.78 microM (brain microdialysates). Of the compounds tested MRZ 2/579, 2/615, 2/632, 2/633, 2/639 and 2/640 had affinities, kinetics and voltage-dependency most similar to those of memantine and had good therapeutic indices against MES-induced convulsions. We predict that these amino-alkyl-cyclohexanes, which all had methyl substitutions at R1, R2, and R5, at least one methyl or ethyl at R3 or R4 and a charged amino-containing substitution at R6,

could be useful therapeutics in a wide range of CNS disorders proposed to involve disturbances of glutamatergic transmission.

Petrulis JR, Bunce NJ. **Competitive inhibition by inducer as a confounding factor in the use of the ethoxyresorufin-O-deethylase (EROD) assay to estimate exposure to dioxin-like compounds.** Toxicol Lett 1999;105(3):251-60.
The ethoxyresorufin-O-deethylase (EROD) assay has been extensively used in whole animals and in cell culture as a biomarker of exposure to environmental contaminants such as dioxin-like compounds (DLCs). This paper addresses two controversial phenomena that arise when DLCs are examined by the EROD assay. Firstly, the maximum level of induced EROD activity varies with the identity of the inducing compound; secondly, the induced EROD activity reaches a concentration-dependent maximum level that is followed by an apparent reduction in activity when the concentration of inducer is further increased. These phenomena are completely explained by competitive inhibition of the EROD enzyme-substrate reaction by the dioxin-like compound. A kinetic model explains the biphasic appearance of EROD induction curves as a function of a compound's binding affinity with the Ah receptor (Kd) and its binding affinity to CYP 1A1 (Ki) which results in inhibition of the EROD enzyme-substrate reaction. These results limit the reliability of the information obtained from calibration curves of EROD activity versus concentration of a standard DLC such as 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Pipkin JL, Hinson WG, James SJ, Shaddock JG, Lyn-Cook LE, Feuers RJ, Morris SM, Tolleson WH, Casciano DA. **The relationship of p53 and stress proteins in response to bleomycin and retinoic acid in the p53 heterozygous mouse.** Biochim Biophys Acta 1999;1450(2):164-76.
A single, i.p. dose of bleomycin was administered simultaneously with [35S]methionine to 4-month-old p53 wild type (+/+) and p53 heterozygous (+/-) C57BL/6 mice. Following a period of 3.5 h from dosing, the bone marrow nuclei were examined by two-dimensional PAGE and fluorography for induction of stress proteins (sps). Eight sps ranging from 22000 to 100000 Mr were synthesized in p53+/- and p53+/+ mice following elicitation by bleomycin. No quantitative or qualitative differences were observed in sp expression in these two groups of animals. In a second experiment, three doses of retinoic acid were given i.p. to p53+/- and p53+/+ mice over a 36 h period. The p53 isoforms in bone marrow nuclei from these mice were analyzed by PAGE for incorporation of [35S]methionine following retinoic acid injections. Quantitative and qualitative alterations in p53 isotypes were substantially increased in p53+/+ as compared with p53+/- mice. The increased complexity in the synthesis patterns in both groups of dosed mice consisted of additional isoforms possessing more acidic isoelectric values. In an in vitro binding assay, individual p53 isoforms demonstrated varying degrees of association with sps 25a, 70i, 72c and 90 which was consistently greater in p53+/+ mice. Both the synthesis and binding of isoforms were greater in G1 than in S+G2 phase, in both groups of animals, reflecting a cell cycle regulated mechanism for these events. Collectively, these data implied that the synthesis and the binding characteristics of p53 isoforms with sps were enhanced in the p53+/+ mice relative to the p53+/- mouse; however, sp labeling was not affected by p53 genotype.

Portnoy ME, Rosenzweig AC, Rae T, Huffman DL, O'Halloran TV, Culotta VC. **Structure-function analyses of the ATX1 metallochaperone.** J Biol Chem 1999;274(21):15041-5.
Saccharomyces cerevisiae Atx1p represents a member of the family of metallochaperone molecules that escort copper to distinct intracellular targets. Atx1p specifically delivers copper to the Ccc2p copper

transporter in the Golgi. Additionally, when overproduced, Atx1p substitutes for superoxide dismutase 1 in preventing oxidative damage; however the mechanistic overlap between these functions is unresolved. The crystal structure of Atx1p has been solved recently. By examining a surface electrostatic potential distribution, multiple conserved lysines are revealed on one face of Atx1p. An additional conserved lysine (Lys65) lies in close proximity to the metal binding site. Through site-directed mutagenesis, residues in the metal binding region including Lys65 were found to be necessary for both copper delivery to Ccc2p and for Atx1p antioxidant activity. Copper trafficking to Ccc2p also relied on the lysine-rich face of Atx1p. Surprisingly however, elimination of these lysines did not inhibit the antioxidant activity of Atx1p. We provide evidence that Atx1p does not suppress oxidative damage by a metallochaperone mechanism but may directly consume superoxide. Purified Cu-Atx1p reacts noncatalytically with superoxide anion in vitro. We conclude that the copper-trafficking and antioxidant functions of Atx1p arise from chemically and structurally distinct attributes of this metallochaperone.

Potolicchio I, Festucci A, Hausler P, Sorrentino R. **HLA-DP molecules bind cobalt: a possible explanation for the genetic association with hard metal disease.** Eur J Immunol 1999;29(7):2140-7.
Metal dust inhalation induces an interstitial lung disease which may progress to pulmonary fibrosis (hard metal disease, HMD). Cobalt is believed to be the pathogenic agent of HMD. A strong genetic association of HMD with some HLA-DP alleles has been reported although the role of these molecules in the occurrence of the fibrotic disorder remains unclear. A possible explanation of these findings is that HLA-DP but not other HLA class II molecules can bind cobalt. This could have as a consequence an HLA-DP-mediated specific activation of the immune system. To test this hypothesis, we have set up an in vitro binding assay using 57Co and purified HLA-DP and -DR molecules. The results indicate that HLA-DP but not HLA-DR molecules bind cobalt. Moreover, the presence of HLA-DP Glu beta69, which is associated with susceptibility to HMD, determines a higher metal uptake. Molecular modelling of HLA-DP2 molecules places the Glu beta69 residue in a position relevant in determining peptide specificity. The possibility that binding of cobalt by HLA-DP molecules can interfere with their antigen presenting functions is discussed.

Prahalad AK, Soukup JM, Inmon J, Willis R, Ghio AJ, Becker S, Gallagher JE. **Ambient air particles: effects on cellular oxidant radical generation in relation to particulate elemental chemistry.** Toxicol Appl Pharmacol 1999;158(2):81-91.
Epidemiologic studies have reported causal relationships between exposures to high concentrations of ambient air particles (AAP) and increased morbidity in individuals with underlying respiratory problems. Polymorphonuclear leukocytes (PMN) are frequently present in the airways of individuals exposed to particles. Upon particulate stimulation the PMN may release reactive oxygen species (ROS), which can result in tissue damage and injury. In this study a wide range of AAP samples from divergent sources (1, natural dust; 2, oil fly ash; 2, coal fly ash; 5, ambient air; and 1, carbon black) were analyzed for elemental content and solubility in relation to their ability to generate ROS. Elemental analyses were carried out in AAP and dH(2)O-washed AAP using energy dispersive x-ray fluorescence (XRF). Percent of sample mass accounted for by XRF-detectable elements was 1.2% (carbon black); 22-29% (natural dust and ambient air particles); 13-22% (oil fly ash particles); 28-49% (coal fly ash particles). The major proportion of elements in most of these particles were aluminosilicates and insoluble iron, except oil-derived fly ash particles in which soluble vanadium and nickel were in highest concentrations, consistent

with particle acidity as measured in the supernatants. Human blood-derived monocytes and PMN were exposed to AAP and dH(2)O-washed particles, and generation of ROS was determined using luminol-enhanced chemiluminescence (LCL) assay. All the particles induced chemiluminescence response in the cells, except carbon black. The oxidant response of monocytes induced by AAP (with the exception of oil fly ash particles) was less than the response elicited by PMN. The LCL response of PMN in general increased with all washed particles, with oil fly ash (OFA) and one urban air particle showing statistically significant ($p < 0.05$) differences between dH(2)O-washed and unwashed particles. The LCL activity in PMN induced by both particles and dH(2)O-washed particles was significantly correlated with the insoluble Si, Fe, Mn, Ti, and Co content of particles ($p < 0.05$). No relationship between LCL activity in PMN and soluble transition metals such as V, Cr, Ni, and Cu was noted. Pretreatment of the particles with a metal ion-chelator, deferoxamine, did not affect LCL in PMN, suggesting that metal ions are not related to the induction of LCL in PMN. Particulate S content and acidity of the particles as measured in the supernatants did not relate to LCL activity in PMN. These results point to the possibility that the insoluble constituents of the particles are related to LCL in PMN. Since some of these dusts are capable of depositing in the lungs and can cause infiltration of PMN, the ability to activate those cells may contribute to particulate toxicity. Copyright 1999 Academic Press.

Qu SJ, Fan HZ, Kilinc C, Pownall HJ. **Role of cysteine residues in human plasma phospholipid transfer protein.** J Protein Chem 1999;18(2):193-8.
Phospholipid transfer protein (PLTP) belongs to a family of human plasma lipid transfer proteins that bind to small amphophilic molecules. PLTP contains cysteines at residues 5, 129, 168, and 318. Bactericidal/permeability-increasing protein, which is a member of the same gene family, contains an essential disulfide bond between Cys135 and Cys175; these residues, which correspond to Cys129 and Cys168 in PLTP, are conserved among all known members of the gene family. To identify the importance of these and the remaining cysteine residues to PLTP secretion and activity, each was replaced by a glycine by site-directed mutagenesis. The mutant as well as wild-type PLTP cDNAs were cloned into the mammalian expression vector pSV.SPORT1, and the PLTP cDNAs were transfected to COS-6 cells for expression. PLTP Cys129 --> Gly and PLTP Cys168 --> Gly were secretion incompetent. Neither PLTP mass nor activity was detectable in cell lysates and culture medium. Relative to wild-type PLTP, PLTP Cys5 --> Gly and PLTP Cys318 --> Gly exhibited similar specific activities but partially impaired PLTP synthesis and secretion. Intracellular PLTP appeared as two bands of 75 and 51 kDa corresponding to reported molecular masses for the glycosylated and nonglycosylated forms. The specific activities of PLTP Cys5 --> Gly and PLTP Cys318 --> Gly were similar in the cell lysates and medium, suggesting that glycosylation does not affect transfer activity.

Quick DJ, Shuler ML. **Use of in vitro data for construction of a physiologically based pharmacokinetic model for naphthalene in rats and mice to probe species differences.** Biotechnol Prog 1999;15(3):540-55.
A physiologically based pharmacokinetic (PBPK) model with five tissue groups (lung, liver, fat, richly perfused, and poorly perfused tissues plus venous and arterial blood compartments) has been developed from in vitro data and models of primary cell cultures for naphthalene toxicity in mice and rats. It extends a previous naphthalene PBPK model (Sweeney et al., 1996) and demonstrates a possible approach to a predictive mathematical model that requires minimal animal data. Naphthalene

metabolism was examined after four exposure routes (intraperitoneal injection (ip), intravenous injection (iv), ingestion (po), and inhalation). Naphthalene and its primary metabolite, naphthalene oxide, are consumed by enzymes in pulmonary and hepatic tissues (cytochrome P450 monooxygenases, epoxide hydrolase, and glutathione-S-transferase). Additionally, the nonenzymatic reactions of naphthalene oxide in all tissues and in blood are included in the model. Kinetic constants for the model were derived primarily from cell fraction and primary cell culture incubations presented in the literature. The mouse model accurately predicts glutathione (GSH) and covalent naphthalene oxide-protein binding levels after a range of ip doses, and the rat model provides excellent estimates for mercapturate excretion following po doses; but neither model simulates well naphthalene blood concentrations after low iv doses. Good prediction of in vivo response using only in vitro data for parameter estimation (except for epoxide-protein binding rates) suggests that the assumed molecular description is a plausible representation of the underlying mechanisms of toxicity. Mice and rats show significant species differences in response to naphthalene. The model results suggest that species differences in toxicity may be explained, in part, by the lower overall rate of enzyme activities in the rat cells. Lower enzyme activities in the rat result in out-of-phase GSH minima in hepatic and lung compartments, while the simultaneous occurrence of these minima in mice results in higher naphthalene oxide concentrations, thereby allowing formation of more metabolites (e.g., covalent binding to proteins) that may be toxic.

Ramchandani VA, Bolane J, Li TK, O'Connor S. **A physiologically-based pharmacokinetic (PBPK) model for alcohol facilitates rapid BrAC clamping.** Alcohol Clin Exper Res 1999;23(4):617-23.
BIOSIS COPYRIGHT: BIOL ABS. Alcohol clamping is a technique that maintains a constant breath alcohol concentration (BrAC) for prolonged intervals, thereby reducing experimental variance in the time course of organ exposure to alcohol, when compared with oral alcohol administration paradigms. The technique employs an intravenous (iv) infusion of an ethanol solution at a rate that is intermittently adjusted based on real-time BrAC measurements. In earlier studies, when the clamped state was induced with an oral ethanol loadi ng dose with a preprogrammed infusion rate profile. A three-compartment physiologically-based pharmacokinetic (PBPK) model for ethanol was constructed, then tailored to each subject using individualized estimates of model parameters. The model was used to compute the infusion-rate profile that would produce the desired time course of BrAC when infused in the corresponding subject. The two clamping methods were compared in a two-session crossover study in 20 healthy young subjects (10 males, 10 f.

Rego AC, Santos MS, Oliveira CR. **Influence of the antioxidants vitamin E and idebenone on retinal cell injury mediated by chemical ischemia, hypoglycemia, or oxidative stress.** Free Radic Biol Med 1999;26(11-12):1405-17.
A role for the antioxidants vitamin E and idebenone in decreasing retinal cell injury, after metabolic inhibition induced by chemical ischemia and hypoglycemia, was investigated and compared with oxidative stress conditions. Preincubation of the antioxidants, vitamin E (20 microM) and idebenone (10 microM), effectively protected from retinal cell injury after oxidative stress or hypoglycemia, whereas the protection afforded after postincubation of both antioxidants was decreased. Delayed retinal cell damage, mediated by chemical ischemia, was attenuated at 10 or 12 h postischemia, only after exposure to the antioxidants during all the experimental procedure. An antagonist of the N-methyl-D-aspartate (NMDA) receptors, an inhibitor of nitric oxide synthase (NOS) or a blocker of L-type Ca2+ channels

were ineffective in reducing cell injury induced by chemical ischemia, hypoglycemia or oxidative stress. Oxidative stress and hypoglycemia increased (about 1.2-fold) significantly the fluorescence of the probe DCFH2-DA, that is indicative of intracellular ROS formation. Free radical generation detected with the probe dihydrorhodamine 123 (DHR 123) was enhanced after oxidative stress, chemical ischemia or hypoglycemia (about 2-fold). Nevertheless, the antioxidants vitamin E or idebenone were ineffective against intracellular ROS generation. Cellular energy charge decreased greatly after chemical ischemia, was moderately affected after hypoglycemia, but no significant changes were observed after oxidative stress. Preincubation with vitamin E prevented the changes in energy charge upon 6 h posthypoglycemia. We can conclude that irreversible changes occurring during chemical ischemia mainly reflect the alterations taking place at the ischemic core, whereas hypoglycemia situations may reflect changes occurring at the penumbra area, whereby vitamin E or idebenone may help to increase cell survival, exerting a beneficial neuroprotective effect.

Renglin A, Harmala-Brasken AS, Eriksson JE, Onfelt A. **Mitotic aberrations induced by carbaryl reflect tyrosine kinase inhibition with coincident up-regulation of serine/threonine protein phosphatase activity: implications for coordination of karyokinesis and cytokinesis.** Mutagenesis 1999;14(3):327-33.
The insecticide carbaryl and its metabolite 1-naphthol cause partial uncoupling of karyokinesis and cytokinesis in V79 Chinese hamster fibroblasts; karyokinesis is blocked in metaphase, the microtubules of the spindle depolymerize and the chromosomes and spindle remnants become displaced to the periphery of the cell. A high frequency of these disturbed cells elongate and a smaller fraction initiate a cleavage furrow. Here, we attempt to determine the potential targets for carbaryl and 1-naphthol in cytokinesis-specific signalling, led by the fact that the potential protein phosphatase inhibitor 1-naphthyl phosphate was previously identified in treated cells. We found that the typical cytological pattern induced by carbaryl and 1-naphthol could be obtained with tyrphostins, specific tyrosine kinase inhibitors, indicating that the carbaryl-induced effects could be due to tyrosine kinase inhibition. This was confirmed by tyrosine kinase assays showing that carbaryl, 1-naphthol and 2-naphthol were equally efficient at inhibiting tyrosine kinase activity as tyrphostin B44(-). As tyrosine kinases can act as regulatory factors in determining dephosphorylation rates, the activities of type-1 (PP1) and type-2A (PP2A) serine/threonine protein phosphatases were also determined. There was a clear up-regulation of the overall PP1/PP2A activities in cells treated with carbaryl, 1-naphthol or tyrphostin B44(-). This stimulation was shown to be indirect because these compounds had no effect on the activity of purified human PP1 in the test tube. 2-Naphthol, which has been found to be less efficient with regard to displacement of chromatin, did not cause up-regulation, but a significant decrease in PP1/PP2A activity. We suggest that a net decrease in tyrosine kinase activity in combination with a net increase in PP1/PP2A activity is a precondition for cell elongation and cytokinesis in mammalian cells and that the corresponding enzymes are targets in the network of activities serving to coordinate karyokinesis and cytokinesis.

Rentschler S, Linn H, Deininger K, Bedford MT, Espanel X, Sudol M. **The WW domain of dystrophin requires EF-hands region to interact with beta-dystroglycan.** Biol Chem 1999;380(4):431-42.
Skeletal muscle dystrophin is a 427 kDa protein thought to act as a link between the actin cytoskeleton and the extracellular matrix. Perturbations of the dystrophin-associated complex, for example, between

dystrophin and the transmembrane glycoprotein beta-dystroglycan, may lead to muscular dystrophy. Previously, the cysteine-rich region and first half of the carboxy-terminal domain of dystrophin were shown to interact with beta-dystroglycan through a stretch of fifteen amino acids at the carboxy-terminus of beta-dystroglycan. This region of dystrophin implicated in binding beta-dystroglycan contains four modular protein domains: a WW domain, two putative Ca2+-binding EF-hand motifs, and a putative zinc finger ZZ domain. The WW domain is a globular domain of 38-40 amino acids with two highly conserved tryptophan residues spaced 20-22 amino acids apart. A subset of WW domains was shown to bind ligands that contain a Pro-Pro-x-Tyr core motif (where x is any amino acid). Here we elucidate the role of the WW domain of dystrophin and surrounding sequence in binding beta-dystroglycan. We show that the WW domain of dystrophin along with the EF-hand motifs binds to the carboxy-terminus of beta-dystroglycan. Through site-specific mutagenesis and in vitro binding assays, we demonstrate that binding of dystrophin to the carboxy-terminus of beta-dystroglycan occurs via a beta-dystroglycan Pro-Pro-x-Tyr core motif. Targeted mutagenesis of conserved WW domain residues reveals that the dystrophin/beta-dystroglycan interaction occurs primarily through the WW domain of dystrophin. Precise mapping of this interaction could aid in therapeutic design.

Resjo S, Oknianska A, Zolnierowicz S, Manganiello V, Degerman E. **Phosphorylation and activation of phosphodiesterase type 3B (PDE3B) in adipocytes in response to serine/threonine phosphatase inhibitors: deactivation of PDE3B in vitro by protein phosphatase type 2A.** Biochem J 1999;341(Pt 3):839-45.
Phosphodiesterase type 3B (PDE3B) has been shown to be activated and phosphorylated in response to insulin and hormones that increase cAMP. In order to study serine/threonine protein phosphatases involved in the regulation of rat adipocyte PDE3B, we investigated the phosphorylation and activation of PDE3B in vivo in response to phosphatase inhibitors and the dephosphorylation and deactivation of PDE3B in vitro by phosphatases purified from rat adipocyte homogenates. Okadaic acid and calyculin A induced dose- and time-dependent activation of PDE3B. Maximal effects were obtained after 30 min using 1 microM okadaic acid (1.8-fold activation) and 300 nM calyculin A (4-fold activation), respectively. Tautomycin and cyclosporin A did not induce activation of PDE3B. Incubation of adipocytes with 300 nM calyculin A inhibited protein phosphatase (PP) 1 and PP2A completely. Okadaic acid (1 microM) reduced PP2A activity by approx. 50% but did not affect PP1 activity, and 1 microM tautomycin reduced PP1 activity by approx. 60% but PP2A activity by only 11%. This indicates an important role for PP2A in the regulation of PDE3B. Furthermore, rat adipocyte PDE3B phosphatase activity co-purified with PP2A but not with PP1 during MonoQ chromatography. As compared with insulin, okadaic acid and calyculin A induced phosphorylation of PDE3B by 2.8- and 14-fold respectively, whereas tautomycin and cyclosporin A had no effect. Both calyculin A and okadaic acid induced phosphorylation on serine 302, the site known to be phosphorylated on PDE3B in response to insulin and isoproterenol (isoprenaline), as well as on sites not identified previously. In summary, PP2A seems to be involved in the regulation of PDE3B in vivo and can act as a PDE3B phosphatase in vitro. In comparison with insulin, calyculin A induced a dramatic activation of PDE3B and both calyculin A and okadaic acid induced phosphorylation on additional sites, which could have a role in signalling pathways not yet identified.

Ridgley EL, Xiong ZH, Ruben L. **Reactive oxygen species activate a Ca2+-dependent cell death**

pathway in the unicellular organism Trypanosoma brucei brucei. Biochem J 1999;340(Pt 1):33-40.
Here we examine a cell death process induced by reactive oxygen species (ROS) in the haemoflagellate Trypanosoma brucei brucei. Ca2+ distribution in cellular compartments was measured with stable transformants expressing aequorin targeted to the cytosol, nucleus or mitochondrion. Within 1.5 h of ROS production, mitochondrial Ca2+ transport was impaired and the Ca2+ barrier between the nuclear envelope and cytosol was disrupted. Consequently the mitochondrion did not accumulate Ca2+ efficiently in response to an extracellular stimulus, and excess Ca2+ accumulated in the nucleus. The terminal transferase deoxytidyl uridine end labelling assay revealed that, 5 h after treatment with ROS, extensive fragmentation of nuclear DNA occurred in over 90% of the cells. Permeability changes in the plasma membrane did not occur until an additional 2 h had elapsed. The intracellular Ca2+ buffer, EGTA acetoxymethyl ester, prevented DNA fragmentation and prolonged the onset of changes in cell permeability. Despite some similarities to apoptosis, nuclease activation was not a consequence of caspase 3, caspase 1, calpain, serine protease, cysteine protease or proteasome activity. Moreover, trypanosomes expressing mouse Bcl-2 were not protected from ROS even though protection from mitochondrial dysfunction and ROS have been reported for mammalian cells. Overall, these results demonstrate that Ca2+ pathways can induce pathology in trypanosomes, although the specific proteins involved might be distinct from those in metazoans.

Romero MP, Osuna C, Garcia-Perganeda A, Carrillo-Vico A, Guerrero JM. **The pineal secretory product melatonin reduces hydrogen peroxide-induced DNA damage in U-937 cells.** J Pineal Res 1999;26(4):227-35.
Melatonin, the chief secretory product of the pineal gland, is a potent and efficient endogenous radical scavenger. Thus, melatonin was shown to protect different biomolecules, such as DNA, membrane lipids, and cytosolic proteins, from oxidative damage induced by oxygen-derived free radicals. In order to study the protective role of melatonin in hydrogen peroxide (H2O2)-induced DNA damage, U-937 cells were treated with different concentrations of H2O2, either in the presence or absence of melatonin, and DNA damage was assessed using the cytokinesis-block micronucleus technique. Melatonin diminished H2O2-induced micronuclei production both in short and long treatments. Additionally, melatonin concentrations higher than 1 microM were capable of protecting cells from spontaneous micronuclei production. These data suggest that melatonin, an endogenous antioxidant and nontoxic compound, may have an important role in protecting cells from genetic damage due to free radicals, supporting the idea of this hormone as a possible therapeutic agent in preventing aging and age-related diseases.

Ropiquet F, Huguenin S, Villette JM, Ronfle V, Le Brun G, Maitland NJ, Cussenot O, Fiet J, Berthon P. **FGF7/KGF triggers cell transformation and invasion on immortalised human prostatic epithelial PNT1A cells.** Int J Cancer 1999;82(2):237-43.
Fibroblast growth factor 7 (FGF7/KGF) is synthesized exclusively by fibroblasts in normal tissues; it acts as a potent mitogen on epithelial cells, through interaction with the FGF7-specific receptor FGFR2/IIIb. To examine the importance of this growth factor both to prostate physiology and to prostate-cancer progression, we have tested the exogenous effect of FGF7. Thus, by mimicking the paracrine pathway (on proliferation, growth in soft agar and invasion) on the human prostatic epithelial cell line PNT1A positively checked for FGFR2/IIIb expression, FGF7 significantly enhanced cell proliferation at an

optimal concentration of 7.5 x 10(-11) M, but no significant invasion or growth in soft agar were observed. To confirm FGF7 properties on human prostatic epithelial cells, we constitutively expressed FGF7 by transfecting PNT1A cells with FGF7-cDNA. The FGF7-transfected clones, PNT1A/ FGF7-T5 and PNT1A/FGF7-T6, were stable and expressed FGF7. Analysis of the FGF7-autocrine loop on the non-tumorigenic epithelial cells PNT1A showed acquired invasive potential in in vitro extracellular-matrix migration assays, specifically inhibited by an FGF7-neutralizing antibody, and over-expressed factors implicated in the migration process: the metalloproteinase MMP-1 and the plasminogen activator uPA. Taken together, these results demonstrate a role for FGF7 in triggering invasion of human prostatic epithelial cells. Furthermore, these FGF7-transfected clones exhibited functional and physiological differences from the original PNT1A cell line: anchorage-independent growth, growth in serum-free media and increased proliferation. These data confirm the oncogenic function of FGF7 in prostate progression potentially acting through paracrine and/or autocrine regulatory pathways.

Rosenberger SF, Gupta A, Bowden GT. **Inhibition of p38 MAP kinase increases okadaic acid mediated AP-1 expression and DNA binding but has no effect on TRE dependent transcription.** Oncogene 1999;18(24):3626-32.
By performing in vitro kinase assays we found in papilloma producing 308 mouse keratinocytes that okadaic acid elevated activities of extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinases (MAPKs). This okadaic acid mediated activation of MAP kinases correlated with increased AP-1 binding to a consensus TPA responsive element (TRE) and elevated TRE dependent transcription. To determine the role of p38 MAP kinases in these processes we employed the specific p38 MAP kinase inhibitor SB 203580. Using orthophosphate labeling we showed a decrease in phosphorylation of MAPK activated protein kinase-2 (MAPKAP-K2) indicating reduced activity of p38 MAPKs utilizing this kinase as substrate. In contrast, we found that SB 203580 raised activities of ERK-1/2 and JNKs. Electrophoretic mobility shift assays revealed an increase in TRE binding activity in response to SB 203580 most likely resulting from increased expression of the major TRE binding components JunD and FosB as indicated by Western blot analyses. Increased TRE DNA binding failed to lead to increased transactivation correlating with the inability of SB 203580 to increase phosphorylation of these AP-1 proteins. These data indicate that SB 203580 sensitive p38 MAP kinases are not involved in okadaic acid mediated increases in TRE DNA binding and transactivation.

Sakata A, Kuwahara K, Ohmura T, Inui S, Sakaguchi N. **Involvement of a rapamycin-sensitive pathway in CD40-mediated activation of murine B cells in vitro.** Immunol Lett 1999;68(2-3):301-9.
Activation of resting B cells requires an initial triggering of the B cell antigen receptor (BCR) and secondary stimuli through various cytokine receptors and B cell activation molecules including CD40. We found that activation of B cells through CD40 is selectively inhibited by an immunosuppressant drug, rapamycin. This effect of rapamycin on anti-CD40-mediated activation of B cells was observed using three different in vitro assays. Rapamycin suppressed the anti-CD40-induced proliferation of splenic B cells, suppressed differentiation to surface IgMhigh/IgDlow B cells, and inhibited an anti-CD40-mediated prevention of apoptosis induced by BCR cross-linkage of WEHI-231 cells. We next examined several known CD40 signal transduction pathways to identify the target of rapamycin in stimulated B cells. Rapamycin did not inhibit the activation of c-Jun N-terminal kinases (JNKs) induced

by anti-CD40 stimulation nor the activation of immediate nuclear transcription factors of NF-kappaB. Therefore, rapamycin affects a novel element of the CD40 signal transduction pathway which influences the proliferation, differentiation, and prevention of apoptosis of B cells.

Sandhoff TW, MClean MP. **Repression of the rat steroidogenic acute regulatory (StAR) protein gene by PGF2alpha is modulated by the negative transcription factor DAX-1.** Endocrine 1999;10 (1):83-91.
The steroidogenic acute regulatory protein (StAR) is thought to mediate the rapid increase in steroid hormone biosynthesis by facilitating cholesterol transport to the inner mitochondrial membrane. Recent studies indicate that StAR gene expression is enhanced by gonadotropins, whereas prostaglandin F2alpha (PGF2alpha) appears to suppress both basal and gonadotropin-stimulated StAR mRNA levels. While studies have demonstrated that steroidogenic factor 1 (SF-1) mediates transcriptional activation of the StAR gene, the mechanism for the reduction in StAR expression requires analysis. Recent studies have shown that DAX-1 (Dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X-chromosome, gene-1), a negative transcription factor, inhibits transcription of reporter genes in vitro. To determine whether DAX-1 could negatively regulate expression of the StAR gene, approx 2 kb of the rat StAR promoter was linked to a luciferase reporter gene (creating p-1862 StAR) and cotransfected into Y1 adrenal tumor cells and HTB9 human bladder carcinoma cells with vectors which encode DAX-1 and SF-1. Luciferase levels were significantly increased in both cell types when SF-1 was present. In contrast, when DAX-1 was cotransfected with the StAR promoter, Y1 adrenal and HTB9 cell luciferase activities were reduced to levels that were 57% and 24% of basal promoter levels, respectively. Furthermore, when dibutyryl-cAMP (dbcAMP) was added to the DAX-1 expressing cells, cAMP responsiveness was repressed 50% and 75% in Y1s and HTB9s respectively, relative to the non-DAX-1 expressing dbcAMP-treated cells. The inhibition of StAR gene transcription by DAX-1 was dose-dependent reducing transcription to 6% of control levels. Consistent with the possibility that PGF2alpha regulates ovarian StAR expression via DAX-1, Western blot analysis indicated a three- and fivefold increase in rat ovarian DAX-1 levels at 2 and 4 h following PGF2alpha injection (250 microg). The increase in DAX-1 protein corresponded to a 50% reduction in StAR mRNA levels concomitant with a 39% reduction in serum progesterone levels. Truncation of the DAX-1 protein at the C-terminal end caused a loss of inhibition of transcriptional activity. Deletion of bp -95 to -50 within the StAR promoter, a proposed DAX-1 binding site, did not alter the ability of wild-type DAX-1 to inhibit transcription. In a mammalian two-hybrid system, cotransfection of DAX-1 and SF-1 caused a 25-fold induction in luciferase activity demonstrating that these proteins interact in the two-hybrid assay. This study is the first to demonstrate that the rat StAR promoter is regulated by DAX-1 and that DAX-1 reduces StAR promoter responsiveness to cAMP. The enhanced level of DAX-1 following PGF2alpha administration is consistent with DAX-1 having a role in controlling both basal, gonadotropin-stimulated, and PGF2alpha-mediated StAR gene expression. These results imply that DAX-1 has an important role in regulating ovarian steroidogenesis by repressing StAR transcription.

Sass JO, Zimmermann B, Ruhl R, Nau H. **Effects of all-trans-retinoyl-beta-D-glucuronide and all-trans-retinoic acid on chondrogenesis and retinoid metabolism in mouse limb bud mesenchymal cells in vitro.** Arch Toxicol 1997;71(3):142-50.
Retinoids, derivatives of vitamin A, are essential for many vertebrate functions. Furthermore, several

drugs of this class of compounds are valuable in the treatment of certain forms of skin disorders and cancer. However, the therapeutic application of retinoids is limited by their teratogenic potency. The limbs are important sites of retinoid-induced malformations in rodents. Therefore, organoid cultures of limb bud mesenchymal cells have been established for screening of the teratogenic potency of retinoids. We have now applied this system to compare the effects of all-trans-retinoyl-beta-D-glucuronide (all-trans-RAG) with those of all-trans-retinoic acid (all-trans-RA) on chondrogenesis, as assessed by the Alcian blue binding assay and by electron microscopic evaluation including quantitative morphometric analysis. First data of retinoid toxicokinetics in the culture media as well as retinoid concentrations in the cultured mesenchymal limb bud cells were established. While all-trans-RA inhibited chondrogenesis at 10(-7) M by ca. 50%, tenfold higher concentrations of all-trans-RAG were necessary to obtain the same effect. This difference reflects the ratio of RA isomers which were found in the medium after incubation with either all-trans-RAG or all-trans-RA. A pulse experiment (10(-5) M all-trans-RAG or all-trans-RA for the first 2 h of a 6-day incubation period) demonstrated inhibition of chondrogenesis with all-trans-RA, but not with all-trans-RAG. The data indicate that RAG inhibits chondrogenesis upon hydrolysis to RA. Surprisingly, the rather polar RAG isoforms were extensively accumulated in the limb bud mesenchymal cells when compared to the medium. Both all-trans-RAG and all-trans-RA also induced a large increase of retinyl ester concentrations in the chondrocytes compared to vehicle-treated cells. This finding further supports a recent suggestion that RA regulates retinol metabolism via feedback inhibition of retinol oxidation and stimulation of the esterification of retinol.

Satoh T, Suzuki S, Kawai N, Nakamura T, Hosokawa M. **Toxicological significance in the cleavage of esterase-beta-glucuronidase complex in liver microsomes by organophosphorus compounds.** Chem Biol Interact 1999;119-120:471-8.
Egasyn is an accessory protein of beta-glucuronidase (beta-G) in the liver microsomes. Liver microsomal beta-G is stabilized within the luminal site of the microsomal vesicles by complexation with egasyn which is one of the carboxylesterase isozymes. We investigated the effects of organophosphorus compounds (OPs) such as insecticides on the dissociation of egasyn-beta-glucuronidase (EG) complex. The EG complex was easily dissociated by administration of OPs, i.e. fenitrothion, EPN, phenthionate, and bis-beta-nitrophenyl phosphate (BNPP), and resulting beta-G dissociated was released into blood, leading to the rapid and transient increase of plasma beta-G level with a concomitant decrease of liver microsomal beta-G level. In a case of phenthionate treatment, less increase in plasma beta-G level was observed, as compared with those of other OPs. This may be explained by the fact that phenthionate was easily hydrolyzed by carboxylesterase. Similarly, carbamate insecticides such as carbaryl caused rapid increase of plasma beta-G level. In contrast, no significant increase of plasma beta-G level was observed when pyrethroid insecticides were administered to rats. This is due to the fact that pyrethroids such as phenthrin and allethrin were easily hydrolyzed by A-esterase as well as carboxylesterase. On the other hand, addition of OPs to the incubation mixture containing liver microsomes caused the release of beta-G from microsomes to the medium. From these in vivo and in vitro data, it is concluded that increase of the plasma beta-G level after OP administration is much more sensitive biomarker than cholinesterase inhibition to acute intoxication of OPs and carbamates.

Schmider J, Von Moltke LL, Shader RI, Harmatz JS, Greenblatt DJ. **Extrapolating in vitro data on drug metabolism to in vivo pharmacokinetics: evaluation of the pharmacokinetic interaction**

between amitriptyline and fluoxetine. Drug Metab Rev 1999;31(2):545-60.
Recently, models have been proposed to extrapolate in vitro data on the influence of inhibitors on drug metabolism to in vivo decrement in drug clearance. Many factors influence drug clearance such as age, gender, habits, diet, environment, liver disease, heredity, and other drugs. In vitro investigation of hepatic cytochrome P450 activity has generally centered on genetic influences and interactions with other drugs. This group of enzymes is involved in many, although not all, drug interactions. The interaction of amitriptyline and fluoxetine is an example. Of the different in vitro paradigms, interaction studies utilizing human liver microsomal preparations have proved to be the most generally applicable for in vitro scaling models. Assuming Michaelis-Menten conditions and applying nonlinear regression, a hybrid inhibition constant (Ki) can be generated that allows classification of the inhibitory potency of an inhibitor toward a specific reaction. This constant is largely independent of the substrate concentration, but in vivo relevance is critically dependent on the inhibitor concentration in the site of metabolic activity, the liver cell cytosol. Many lipophilic drugs are extensively bound to plasma protein but, nonetheless, demonstrate extensive partitioning into liver tissue. This is not compatible with diffusion only of the unbound drug fraction into liver cells. The introduction of a partition factor, based on data from a number of possible sources, provided a reasonable basis for the scaling of in vitro data to in vivo conditions. Many interactions could be reconstructed or predicted with greater accuracy and clinical relevance for interactions such as terfenadine or midazolam and ketoconazole. Even for less marked interactions such as amitriptyline and fluoxetine, this model provides a forecast consistent with the clinically observed range of 22-45% reduction in oral clearance, although this interaction is complicated by the presence of two inhibitors, fluoxetine and norfluoxetine. The concept of in vitro-in vivo scaling is promising and might ultimately yield a fast and more cost-effective screening for drug interactions with reduced human drug exposure and risk.

Sen R, Ghosh S. **Induction of premature mitosis in onion root tip cells blocked in S-phase.** Cell Chromosome Res 1997;20(2):53-5.
BIOSIS COPYRIGHT: BIOL ABS. Onion root tip cells were blocked in S-phase by treating them with 5-amino uracil (5 AU). Premature mitosis was induced in these cells blocked in S-phase by purine derivatives as Caffeine and 2 aminopurine (2-AP). These purine derivatives are thus able to over come S-phase check point both in animal and plant cells. This points to an identical biochemical pathway in plant and animal cells from S-phase to the initiation of mitosis. This pathway and the possible mode of action of caffeine and 2-AP.

Severin SE, Muizhenek EL, Severin ES. **[The targetted delivery of antitumor preparations by using protein vectors]**. Vestn Ross Akad Med Nauk 1999;(5):22-8. (Rus)
The prospects of the endocytosis-mediated targeted delivery of antitumor drugs to the target cells by means of vector proteins, such as nerve growth factor (NGF), epidermal growth factor (EGF), and the oncofetal protein alpha-fetoprotein (AFP), are discussed. The high selectivity and efficiency of antitumor effects of synthetic covalent EGF- and AFP-conjugates with chemical agents (antitumor antibiotics) and antisense oligonucleotides are compared with individual biologically active compounds in in vitro and in vivo animal studies. The molecular mechanisms of action of the above conjugates have been studied. Evidence is given for the fact that it is expedient to use them to overcome multidrug resistance in the clinical setting. The findings are the first important step in designing novel target

antitumor drugs based on biologically active vector proteins showing their effects by receptor-mediated endocytosis.

Sewram V, Raynor MW, Raidoo DM, Mulholland DA. **Coupling SFE to uterotonic bioassay: an on-line approach to analysing medicinal plants.** J Pharm Biomed Anal 1998;18(3):305-18.
Supercritical fluid extraction has been directly coupled on-line to a uterotonic bioassay, using guinea pig uterine smooth muscle in vitro. This technique was developed for the detection of uterotonic compounds present in medicinal plants used during pregnancy to induce or augment labour. The direct passage of CO2 into the muscle chamber led to adiabatic cooling of the physiological fluid and inhibition of muscle contraction. This was alleviated by the construction of a CO2 reduction interface together with the passage of carbogen which aided in the rapid displacement of excess CO2. The on-line system was evaluated with four plants (Clivia miniata (Lindl.) Regel, Ekebergia capensis Sparrm., Grewia occidentalis L. and Asclepias fruticosa L.) that are currently used during pregnancy by some black South African women. Extractions were performed with water modified supercritical CO2. Fractions of supercritical fluid extracts, obtained by sequentially increasing the pressure from 200 to 300 and 400 atm at constant temperature were transferred directly to the muscle chamber to identify the active fractions. The 400 atm extracts of C. miniata, A. fruticosa and E. capensis displayed maximum uterotonic activity while only the 300 atm extract of G. occidentalis induced uterine muscle contraction. This technique proved to be a safe and sensitive method for analyzing medicinal plants that contain uterotonic substances hence assisting in rapidly validating the uterotonic properties and detecting any toxic effects of these extracts.

Slamenova D, Horvathova E, Kosikova B, Ruzekova L, Labaj J. **Detection of lignin biopolymer- and vitamin E-stimulated reduction of DNA strand breaks in H2O2- and MNNG-treated mammalian cells by the comet assay.** Nutr Cancer 1999;33(1):88-94.
In this study the possible protective effects of water-soluble sulfur-free lignin biopolymer and vitamin E (alpha-tocopherol) on DNA in human VH10 cells and hamster V79 cells exposed to H2O2 and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) were investigated. The level of DNA damage (DNA strand breaks) was measured using single-cell gel electrophoresis, i.e., comet assay. Lignin biopolymer and vitamin E exhibited a protective effect against the overall DNA damage induced after H2O2 treatment. If H2O2-treated human cells were incubated for 90 minutes to ligate frank breaks of DNA, two lesion-specific enzymes, endonuclease III and formamidopyrimidine DNA glycosylase (FPG), significantly increased the level of DNA strand breaks originating from oxidized pyrimidines and purines. Preincubation of cells with lignin or vitamin E reduced mainly the level of oxidized pyrimidines. Reduction of oxidized purines was less evident. In addition, lignin biopolymer exhibited a protective effect against MNNG-induced DNA damage, whereas vitamin E exhibited a protective effect only against H2O2-induced DNA damage. These findings suggest that the antioxidant nature of lignin biopolymer enables a reduction of the level of frank breaks and of oxidized DNA bases in H2O2-treated cells, and its adsorptive capacity enables binding of nitroso compounds and reduction of alkylation in MNNG-treated cells.

Smargiassi A, Mutti A. **Peripheral biomarkers and exposure to manganese.** Neurotoxicol 1999;20(2-3):401-6.
Biochemical mechanisms underlying manganese (Mn) toxicity include dopamine (DA) auto-oxidation

and free radical generation with subsequent neuronal damage. A neuroendocrine approach based on the measurement of serum prolactin (PRL) has been proposed to assess the tonic inhibition of pituitary lactotrope cells by the tubero-infundibular DA system. Low level exposure to Mn oxides in industrial settings is associated with a shift in the distribution of serum PRL towards higher levels as compared to matched controls. The follow-up of a small cohort of workers from a ferro-manganese plant showed that the increased prevalence of abnormally high PRL values is stable over time. Although the mechanistic basis for their application is less straightforward, other biochemical markers such as dopamine beta hydroxylase and monoamine oxidase Type B, have also been assessed. Contrary to PRL levels, these markers cannot be recommended to monitor early biochemical effects of manganese exposure at the workplace. Early biochemical events can be modified by genetically determined individual differences. Owing to the possible role of a reduced capacity of glutathione conjugation as a risk factor increasing the susceptibility to the action of free radicals generated in the presence of Mn, the class mu glutathione S transferase (GSTM1) genotype has also been assessed in workers occupationally exposed. However, the GSTM1 null genotype does not appear to play an important role in the susceptibility to biochemical effects of Mn. A logistic model of the dose-response relationship based on urinary Mn as marker of exposure indicates that the benchmark dose corresponds to Mn levels as low as 0.4 microgram/l. This would imply that environmental exposure to Mn may contribute to abnormally high serum PRL in the general population.

Soderlind KJ, Gorodetsky B, Singh AK, Bachur NR, Miller GG, Lown JW. **Bis-benzimidazole anticancer agents: targeting human tumour helicases.** Anticancer Drug Des 1999;14(1):19-36.
Certain DNA minor groove binding agents, distamycin, netropsin, and a series of anticancer bis-benzimidazoles can block DNA helicase activity by binding to duplex DNA at specific base sequences. DNA helicases are crucial to cell DNA replication, transcription and repair because these enzymes separate double-stranded DNA, thereby preparing the strands for enzymatic manipulation. From our studies we have developed a hypothesis that focuses on cellular DNA helicase action as a mechanistic site where these minor groove binders can act. A crucial aspect for modulation of DNA activity by drugs is for specificity and selectivity. A series of DNA-interactive bis-benzimidazole analogues of Hoechst 33258 was also prepared to explore the potential for anticancer activity mediated for certain of the drugs via bioreductive activation by endogenous NADH or NADPH. The biological endpoints examined included intracellular distribution in euoxic and hypoxic conditions observed by fluorescence microscopy; relative efficacy as antimetabolites determined by the MTT [tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay in euoxic and hypoxic conditions; and relative inhibitory activities on human DNA helicase, as determined by degree of dissociation of GC B6486 DNA. The intracellular distribution was unique to each of the test compounds. Compounds V-93 and V-153, the respective semiquinone and quinone derivatives, demonstrated the predicted enhanced cytotoxicity and anti-helicase activities, supporting the concept that preferential binding of DNA at 5'-CG and TG sequences provides a novel approach to anticancer drug development.

Soroceanu L, Manning TJ Jr, Sontheimer H. **Modulation of glioma cell migration and invasion using Cl(-) and K(+) ion channel blockers.** J Neurosci 1999;19(14):5942-54.
Human malignant gliomas are highly invasive tumors. Mechanisms that allow glioma cells to disseminate, migrating through the narrow extracellular brain spaces are poorly understood. We recently

demonstrated expression of large voltage-dependent chloride (Cl(-)) currents, selectively expressed by human glioma cells in vitro and in situ (Ullrich et al., 1998). Currents are sensitive to several Cl(-) channel blockers, including chlorotoxin (Ctx), (Ullrich and Sontheimer; 1996; Ullrich et al; 1996), tetraethylammonium chloride (TEA), and tamoxifen (Ransom and Sontheimer, 1998). Using Transwell migration assays, we show that blockade of glioma Cl(-) channels specifically inhibits tumor cell migration in a dose-dependent manner. Ctx (5 microM), tamoxifen (10 microM), and TEA (1 mM) also prevented invasion of human glioma cells into fetal rat brain aggregates, used as an in vitro model to assess tumor invasiveness. Anion replacement studies suggest that permeation of chloride ions through glioma chloride channel is obligatory for cell migration. Osmotically induced cell swelling and subsequent regulatory volume decrease (RVD) in cultured glioma cells were reversibly prevented by 1 mM TEA, 10 microM tamoxifen, and irreversibly blocked by 5 microM Ctx added to the hypotonic media. Cl(-) fluxes associated with adaptive shape changes elicited by cell swelling and RVD in glioma cells were inhibited by 5 microM Ctx, 10 microM tamoxifen, and 1 mM TEA, as determined using the Cl (-)-sensitive fluorescent dye 6-methoxy-N-ethylquinolinium iodide. Collectively, these data suggest that chloride channels in glioma cells may enable tumor invasiveness, presumably by facilitating cell shape and cell volume changes that are more conducive to migration and invasion.

Soucek P. **Cytochrome P450 destruction by quinones: comparison of effects in rat and human liver microsomes.** Chem Biol Interact 1999;121(3):223-36.
Exposure to benzene was recently reported to lower the cytochrome P450 (CYP) content in phenobarbital-pretreated rats in vivo (Gut et al., Environ. Health Perspect. 104 (1996) 1211-1218). This study followed the ability of quinonic benzene metabolites (catechol, hydroquinone, and benzoquinone) to destroy CYP in liver microsomes from rats pretreated with various inducers and in human liver microsomes. Sensitivity of CYP isoforms to destruction was revealed and the interspecies differences assessed. The spectrophotometric evaluations of the total CYP content, assay of CYP marker activities, and electrophoresis with immunoblotting after incubation of microsomes with quinones revealed that: (1) rat liver CYP activities markedly differed in sensitivity to quinone-mediated destruction in vitro, CYP 1A and 3A being the most sensitive isoforms; (2) differences in OH radicals formation and lipid peroxidation among microsomes from rats pretreated with various CYP inducers were also observed; (3) semiquinone radical formation, OH radical production, and induction of lipid peroxidation did not contribute significantly to CYP destruction by quinones; (4) the main mechanism of CYP destruction is covalent binding of the oxidized quinone form to protein and heme moieties of CYP; (5) quinones, mainly benzoquinone, destroy human CYP isoforms to a much greater extent than rat enzymes and thus humans may be much more susceptible to the deleterious effect of benzene metabolism. In conclusion, it is suggested that CYP destruction may be another consequence of benzene exposure and should be taken into consideration when evaluations of possible health risks are performed.

Steel GJ, Laude AJ, Boojawan A, Harvey DJ, Morgan A. **Biochemical analysis of the Saccharomyces cerevisiae SEC18 gene product: implications for the molecular mechanism of membrane fusion.** Biochemistry 1999;38(24):7764-72.
The SEC18 gene product is 48% identical to mammalian NSF (N-ethylmaleimide-sensitive fusion protein), and both proteins encode cytoplasmic ATPases which are essential for membrane traffic in yeast and mammalian cells, respectively. A wealth of biochemical analysis has led to the description of a

model for the action of NSF; through its interaction with SNAPs (soluble NSF attachment proteins), NSF can associate with SNAP receptors (SNAREs) on intracellular membranes, forming 20S complexes. SNAPs then stimulate the intrinsic ATPase activity of NSF, leading to the disassembly of the 20S complex, which is essential for subsequent membrane fusion. Although this model is based almost entirely on in vitro studies of the original clones of NSF and alpha-SNAP, it is nevertheless widely assumed that this mechanism of membrane fusion is conserved in all eukaryotic cells. If so, the crucial biochemical properties of NSF and SNAPs should be shared by their yeast homologues, Sec18p and Sec17p. Using purified recombinant proteins, we report here that Sec18p can specifically interact not only with Sec17p but also with its mammalian homologue, alpha-SNAP. This interaction leads to a stimulation of Sec18p D1 domain ATPase activity, with kinetics similar to those of alpha-SNAP stimulation of NSF, although differences in temperature and N-ethylmaleimide sensitivity were observed between NSF and Sec18p. Furthermore, Sec18p can interact with synaptic SNARE proteins and can synergize with alpha-SNAP to stimulate regulated exocytosis in mammalian cells. We conclude that the mechanistic properties of NSF and SNAPs are shared by Sec18p and Sec17p, thus demonstrating that the biochemistry of membrane fusion is conserved from yeast to mammals.

Styblo M, Thomas DJ. **Binding of arsenicals to proteins in an in vitro methylation system.** Toxicol Appl Pharmacol 1997 Nov;147(1):1-8.
The dynamics of interactions between rat liver cytosolic proteins and arsenicals were examined in an in vitro methylation system that contained cytosol, glutathione, S-adenosylmethionine, and 1 microM - 73As-arsenite. After incubation at 37 degrees C for up to 90 min, low-molecular-weight components of the assay system (<10 kDa) were removed by ultrafiltration and cytosolic proteins were separated by size-exclusion chromatography on Sephacryl S-300 gel. Five 73As-labeled protein peaks were found in chromatographic profiles. The estimated molecular masses of 73As-labeled proteins eluting in the three earliest peaks were as follows: Vo, >/=1000 kDa; A, 135 kDa; and B, 38 kDa. Peak C eluted immediately before the total volume (VT) of the chromatographic column; peak D eluted after the VT. 73As bound to proteins was released by CuCl treatment and speciated by thin-layer chromatography. Amounts and ratios of inorganic As, methyl As, and dimethyl As associated with cytosolic proteins depended upon the incubation interval. Inorganic As was present in all protein peaks. Methyl As was primarily associated with peaks A and C; dimethyl As was associated with peaks B and C. To examine the effect of valence on the binding of methylarsenicals to cytosolic proteins, trivalent or pentavalent 14C-labeled methyl As or dimethyl As was incubated in an in vitro system designed to minimize the enzymatically catalyzed production of methylated arsenicals. Proteins in peaks A, B, and C bound preferentially trivalent methyl and dimethyl As. Peak D bound either trivalent or pentavalent methyl and dimethyl As. Protein-bound inorganic and methyl As were substrates for the production of dimethyl As in an in vitro methylation system, suggesting a role for protein-bound arsenicals in the biomethylation of this metalloid. Copyright 1997 Academic Press.

Subramaniam N, Treuter E, Okret S. **Receptor interacting protein RIP140 inhibits both positive and negative gene regulation by glucocorticoids.** J Biol Chem 1999;274(25):18121-7.
Recent development in the field of gene regulation by nuclear receptors (NRs) have identified a role for cofactors in transcriptional control. While some of the NR-associated proteins serve as coactivators, the effect of the receptor interacting protein 140 (RIP140) on NR transcriptional responses is complex. In

this report we have studied the effect of RIP140 on gene regulation by the glucocorticoid receptor (GR). We demonstrate that RIP140 antagonized all GR-mediated responses tested, which included activation through classical GRE, the synergistic effects of glucocorticoids on AP-1 and Pbx1/HOXB1 responsive elements, as well as gene repression through a negative GRE and cross-talk with NF-kappaB (RelA). This involved the ligand-binding domain of the GR and did not occur when the GR was bound to the antagonist RU486. The strong repressive effect of RIP140 was restricted to glucocorticoid-mediated responses in as much as it slightly increased signaling through the RelA and the Pit-1/Pbx proteins and only slightly repressed signaling through the Pbx1/HOXB1 and AP-1 proteins, excluding general squelching as a mechanism. Instead, this suggests that RIP140 acts as a direct inhibitor of GR function. In line with a direct effect of RIP140 on the GR, we demonstrate a GR-RIP140 interaction in vitro by a glutathione S-transferase-pull down assay. Furthermore, the repressive effect of RIP140 could partially be overcome by overexpression of the coactivator TIF2, which involved a competition between TIF2 and RIP140 for binding to the GR.

Sun AY, Chen YM. **Extracellular ATP-induced apoptosis in PC12 cells.** Adv Exp Med Biol 1998;446:73-83.
Studies in our laboratory indicate that extracellular ATP (ATP)o may induce cell death by reactive oxygen insults. We have also shown that the Ca(2+)-induced oxidative stress as elicited by ATP may lead to an activation of a specific AP-1 activity. Since early impairment of mitochondria constitutes a critical event of the apoptotic cell death, we have examined whether (ATP)o will affect mitochondrial damage and cell injury by using mitochondrial specific probes, dihydrorhodamine and 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). We have found that (ATP)o induced cell death in a concentration dependent manner by MTT assay. The (ATP)o induced cell death correlated well with the reactive oxygen species (ROS) generation in mitochondria, since (ATP)o enhanced both cell death and ROS production and antioxidant blocked both of these processes. We found (ATP)o treatment led to apoptotic cell death by examining DNA laddering and the TUNEL assay. Interestingly, vitamin C and vitamin E combined treatment appeared to attenuate the (ATP)o-induced apoptosis. Results indicated that (ATP)o may cause oxidative damage of mitochondria leading to apoptotic cell death. Antioxidants may be useful in preventing apoptosis by preventing ROS formation in mitochondria.

Sun JS, Shieh KM, Chiang HC, Sheu SY, Hang YS, Lu FJ, Tsuang YH. **Scavenging effect of benzophenones on the oxidative stress of skeletal muscle cells.** Free Radic Biol Med 1999;26(9-10):1100-7.
Benzophenone is an ultraviolet (UV)-absorbing agent that has been used in industry and medicine for more than 30 years. Consumers of cosmetics and sunscreens containing UV-absorbers are exposed to benzophenones on a daily basis, owing to the widespread use of these compounds. However, the efficacy of these compounds as scavengers of oxidative stress is still not well established. In the present study, we investigate the antioxidative capacity of six sunscreen benzophenone compounds. A primary myoblast culture was mixed in vitro with 100 microM menadione. The cytotoxic effect by menadione-induced oxidative stress was monitored by the lucigenin- or luminol-amplified chemiluminescence, methylthiotetrazole (MTT) assay, and the antioxidative effects of various benzophenone compounds were evaluated. The results showed that the addition of menadione can induce oxidative stress on

myoblasts by superoxide and hydrogen peroxide production, which can be eradicated by superoxide dismutase (SOD) and catalase, respectively, in a dose-dependent mode. The catalase has a protective effect on the cytotoxicity induced by menadione as measured by the MTT assay, while the SOD does not. The selected benzophenones also have a significant scavenging effect on the menadione-induced cell death on the myoblasts. The ortho-dihydroxyl structure and other hydroxy groups in the same ring have a stronger scavenging effect on the superoxide anion on myoblasts; thus, a stable penoxy radical may be formed. The mechanism of this effect remains to be clarified.

Sung M, Park K. **Asp 280 residue is important in the activity of the Escherichia coli leader peptidase.** Exp Mol Med 1999;31(2):64-9.
Leader peptidase is a novel serine protease in Escherichia coli, which catalyzes the cleavage of amino-terminal signal sequences from exported proteins. It is an integral membrane protein containing two transmembrane segments with its carboxy-terminal catalytic domain residing in the periplasmic space. Recently, the x-ray crystal structure of signal peptidase-inhibitor complex showed that Asp 280, a highly conserved consensus sequence of E. coli leader peptidase is the closest charged residue in the vicinity of two catalytic dyad, Ser 90 and Lys 145, and it is likely held in place by a salt bridge to Arg 282. Possible roles of Asp 280 and Arg 282 in the structure-catalytic function relationship were investigated by the site-directed mutagenesis of Asp 280 substituted with alanine, glutamic acid, glycine, or asparagine and of Arg 282 with methionine. All of mutants purified with nickel affinity chromatography were inactive using in vitro assay. It is surprising to find complete lose of activity by an extension of one carbon units in the mutant where Asp 280 is substituted with glutamic acid. These results suggest that Asp 280 and Arg 282 are in a sequence which constitutes catalytic crevice of leader peptidase and are essential for maintaining the conformation of catalytic pocket.

Takeda T, Suzuki S, Nagasawa T, Liu RT, Degroot LJ. **DNA binding affinity of hTRbeta1 mutants as heterodimers with traps from different tissues.** Biochimie 1999;81(4):297-308.
Patients with generalized resistance to thyroid hormone (GRTH) show various organ-specific features, for example mental retardation, growth abnormalities, liver damage, delayed bone age or cardiac disorders. Could this reflect aberrant mutant thyroid hormone receptor beta1 (TRbeta1) heterodimerization with specific TR auxiliary proteins (TRAPs) from different tissues, altering the mutant's ability to transactivate tissue-specific genes? To answer this question, we examined the heterodimerization of TRbeta1 mutants and TRAPs of several rat tissues (cerebrum, cerebellum, liver, heart, lung, spleen, and kidney), and in vitro translated RXRalpha, beta and gamma by electrophoretic gel mobility shift assay (EMSA). Mutant TRbeta1 proteins, synthesized in reticulocyte lysate, were incubated with 32P rat malic enzyme (rME) thyroid hormone response elements (TRE) and nuclear extracts of rat tissues. The TRbeta1 mutants used were Mf (G345R), and GH (R316H). Both have non-detectable T3 binding affinity. GH has weak dominant negative effect and Mf has strong dominant negative effect. Two major bands were observed in EMSA. Cerebrum, cerebellum, lung and liver extracts formed a slower migrating band than a TR homodimer, while kidney extracts formed a faster migrating band, and heart and spleen extracts had both bands. There were no qualitative differences in heterodimerization between TRbeta1wt, and TRbeta1 mutants, when using tissue extracts and DNA in excess ratio to TR. We found that RXRalpha, beta, and gamma were differentially expressed in each rat tissue and formed heterodimer complexes with wild type (WT) TRbeta1. Scatchard analysis of affinity

and capacity of the binding of TR-TRAP heterodimers to response elements was performed by competing with 2.5-, 5-, 10-, 25-, and 250-fold excess non-radiolabeled rME-TRE. When using kidney extract, the DNA binding affinity of heterodimers was significantly decreased both in wild type and mutant TRs, suggesting that the DNA binding affinity of the faster migrating band was lower than that of the slower migrating band. Mutant GH, which causes 'pituitary RTH' and shows weak dominant negative effect, tended to form heterodimers with lower DNA binding affinity than TRbeta1wt with all extracts. Mutant Mf, which has strong dominant negative effect, tended to show higher DNA binding affinity than TRbeta1WT. When the data were pooled for all tissues, GH and Mf were found to form heterodimers with significantly lower, or higher, affinity for TREs than TRbeta1wt. These results indicate that: 1) differences of DNA binding affinity of mutant TR-TRAP heterodimers to response elements in DNA play a part in its reduced or strong dominant negative effect; and 2) differences in formation of heterodimers with TRAPs present in tissues do not appear to explain the apparent tissue-specific and mutant-specific variations seen in RTH.

Tang M, Shen X, Frank EG, O'Donnell M, Woodgate R, Goodman MF. **UmuD'(2)C is an error-prone DNA polymerase, Escherichia coli pol V.** Proc Natl Acad Sci U S A 1999;96(16):8919-24.
The damage-inducible UmuD' and UmuC proteins are required for most SOS mutagenesis in Escherichia coli. Our recent assay to reconstitute this process in vitro, using a native UmuD'(2)C complex, revealed that the highly purified preparation contained DNA polymerase activity. Here we eliminate the possibility that this activity is caused by a contaminating DNA polymerase and show that it is intrinsic to UmuD'(2)C. E. coli dinB has recently been shown to have DNA polymerase activity (pol IV). We suggest that UmuD'(2)C, the fifth DNA polymerase discovered in E. coli, be designated as E. coli pol V. In the presence of RecA, beta sliding clamp, gamma clamp loading complex, and E. coli single-stranded binding protein (SSB), pol V's polymerase activity is highly "error prone" at both damaged and undamaged DNA template sites, catalyzing efficient bypass of abasic lesions that would otherwise severely inhibit replication by pol III holoenzyme complex (HE). Pol V bypasses a site-directed abasic lesion with an efficiency about 100- to 150-fold higher than pol III HE. In accordance with the "A-rule," dAMP is preferentially incorporated opposite the lesion. A pol V mutant, UmuD'(2) C104 (D101N), has no measurable lesion bypass activity. A kinetic analysis shows that addition of increasing amounts of pol III to a fixed level of pol V inhibits lesion bypass, demonstrating that both enzymes compete for free 3'-OH template-primer ends. We show, however, that despite competition for primer-3'-ends, pol V and pol III HE can nevertheless interact synergistically to stimulate synthesis downstream from a template lesion.

Tardif G, Pelletier JP, Dupuis M, Geng C, Cloutier JM, Martel-Pelletier J. **Collagenase 3 production by human osteoarthritic chondrocytes in response to growth factors and cytokines is a function of the physiologic state of the cells.** Arthritis Rheum 1999;42(6):1147-58.
OBJECTIVE: We investigated the response of human osteoarthritic (OA) chondrocytes, in terms of collagenase 3 production, to growth factors and cytokines involved in the anabolism and catabolism of articular cartilage, and explored the major signaling pathways leading to its up-regulation. METHODS: Human OA chondrocytes were treated with the following factors: the proinflammatory cytokine interleukin-1beta (IL-1beta), the growth factors basic fibroblast growth factor (bFGF), platelet-derived growth factor BB (PDGF-BB), parathyroid hormone (PTH), insulin-like growth factor 1 (IGF-1),

transforming growth factor gamma1 (TGFbeta1), and TGFbeta2, the protein kinase (PK) activator antagonists for PKC, PKA, and PKG pathways, and phospholipase A2 and tyrosine kinases, as well as the antiinflammatory cytokines IL-4, IL-10, and IL-13. Collagenase 3 expression and synthesis were determined. Comparison was made with collagenase 1. RESULTS: The human OA chondrocyte population could be divided into 2 categories: the L chondrocytes, showing low collagenase 3 basal synthesis levels and high sensitivity to IL-1beta stimulation; and the H chondrocytes, high collagenase 3 basal synthesis levels and low IL-1beta inducibility. In L chondrocytes, all growth factors stimulated collagenase 3 production. In H chondrocytes, PTH, IGF-1, and TGFbeta had little or no impact; bFGF slightly stimulated it and PDGF-BB showed the same pattern as in the L chondrocytes. The effects of all growth factors, except TGFbeta, on collagenase 1 synthesis followed those of collagenase 3, albeit to a higher degree. Interestingly and unlike collagenase 3, the effects of TGFbeta on collagenase 1 could not be related to the state of the cells, but rather, depended on the isoform. Indeed, TGFbeta2 did not induce collagenase 1 synthesis, whereas TGFbeta1 stimulated it. Among the PK activators tested, phorbol myristate acetate was the strongest inducer, suggesting a major involvement of the PKC pathway. IL-13 inhibited collagenase 3 production, IL-4 had little effect, and IL-10 had none. CONCLUSION: This study shows that collagenase 3 production in human OA chondrocytes depends on the physiologic state of the cell. TGFbeta might be responsible for the change in cells from the L to the H state. Importantly, our in vitro data implicate TGFbeta2 as a possible in vivo agent capable of specifically triggering collagenase 3 production over that of collagenase 1 in OA cartilage.

Tardif R, Charest-Tardif G. **The importance of measured end-points in demonstrating the occurrence of interactions: a case study with methylchloroform and m-xylene.** Toxicol Sci 1999;49 (2):312-7.

Mixed exposures may result in significant changes in one biomarker of exposure without altering another biomarker, and this may have unknown significance in terms of exposure assessment and overall toxicity of the mixture. Results from a previous investigation showed that human exposure to methylchloroform (MC, 400 ppm) and m-xylene (XYL, 200 ppm) during 4 h did not result in any significant effect on blood concentrations of these solvents, suggesting the absence of interaction between MC and XYL. Those results were adequately described by conducting a physiologically-based toxicokinetic (PBTK) modeling of the MC-XYL interaction in humans; however, the model suggested that urinary excretion of MC metabolites would be reduced as a result of combined exposure, whereas that of XYL metabolites would not be modified. An experimental verification of this model prediction was then undertaken with rats. In this study, Sprague-Dawley rats (n, 5) were exposed during 4 h to MC (400 ppm) or XYL (200 ppm), alone or as a mixture. Results showed that combined exposure did not affect the blood concentration of MC whereas that of XYL was increased throughout the 2-h blood collection period following exposure. The excretion of MC metabolites during a period of 48 h following the onset of exposure, i.e., trichloroethanol (TCE: -71%) and trichloroacetic acid (TCA: -73%), were significantly reduced. Methylhippuric acid (MHA) was not affected by co-exposure to MC as expected from the PBTK model forecasts. These results exemplify the use of a priori PBPK modeling for designing interaction studies and choosing appropriate/sensitive end-points for demonstrating the occurrence of potential interactions.

Tate DJ Jr, Miceli MV, Newsome DA. **Zinc protects against oxidative damage in cultured human**

retinal pigment epithelial cells. Free Radic Biol Med 1999;26(5-6):704-13.
This study was undertaken to determine whether bioavailable zinc can influence the effects of oxidative stress on cultured human retinal pigment epithelial (RPE) cells. RPE cells were maintained for 7 d in culture medium containing 14 microM total zinc, or in medium containing 0.55 microM total zinc. After 1 week, MTT assays were performed to determine the relative cytotoxicity of H2O2 or paraquat on RPE cells. Conjugated dienes and thiobarbituric acid reactive substances (TBARS) were measured in RPE cells treated with 0, 0.5 mM H2O2, 10 microM FeSO4 + 0.5 mM H2O2 or 10 microM FeSO4 + xanthine/xanthine oxidase for 24 h or paraquat for 7 d. Oxidized proteins were determined by the formation of carbonyl residues. The antioxidants metallothionein, catalase, superoxide dismutase, and glutathione peroxidase were also measured. The MTT assays showed that zinc protected cultured RPE from the toxicity of H2O2 and paraquat. RPE cells in 0.55 microM zinc medium contained higher levels of TBARS, conjugated dienes and protein carbonyls due to the oxidative stresses, compared to cells in 14 microM zinc. Catalase and MT content were reduced in cells cultured in 0.55 microM zinc medium and were reduced additionally when treated with above stresses. Superoxide dismutase activity increased in 0.55 microM zinc medium in response to these stresses. Our results show RPE cells cultured in zinc-reduced medium are more susceptible to oxidative insult.

Tesarik J, Garrigosa L, Mendoza C. **Estradiol modulates breast cancer cell apoptosis: a novel nongenomic steroid action relevant to carcinogenesis.** Steroids 1999;64(1-2):22-7.
It is known that steroids can induce cell surface receptor aggregation followed by activation of receptor and nonreceptor tyrosine kinases. It has been shown recently that 17beta-estradiol (E2) can stimulate the Src/p21ras/mitogen-activated protein kinase pathway in breast cancer cells, and this effect is supposed to mediate the E2-induced stimulation of breast cancer cell proliferation, possibly via activation of the c-fos and c-jun early genes or of genes involved in cell cycle control. Here we demonstrate the existence of an alternative mechanism of the cancer-promoting effect of E2. Human breast cancer cells (MCF-7) were exposed to the known proapoptotic agent vitamin E succinate (VES), added alone or together with different concentrations of E2. E2 conjugated with bovine serum albumin (E2-BSA), which cannot cross the plasma membrane of living cells, was also used in some experiments to assess whether E2 acted on the cell surface or at intracellular receptors. Apoptosis was analyzed by fluorescence-activated cell sorting after cell staining with propidium iodide and FITC-labeled annexin V. E2 showed a concentration-dependent stimulatory effect on spontaneous apoptosis but inhibited the VES-induced apoptosis. However, effects produced by the same molar concentrations of E2 were different when the hormone was free and when it was used in the form of the E2-BSA conjugate. The effects of E2 and E2-BSA were sensitive to genistein, a tyrosine kinase inhibitor. These data show that E2 modulates apoptosis of breast cancer cells, probably acting both at the cell surface and inside the cells. Tyrosine phosphorylation is involved in the signaling pathways mediating this E2 effect.

Thomet U, Furtmuller R, Sieghart W, Le Hyaric-Almeida M, Rousseau JF, Dodd RH, Venault P, Chapouthier G, Sigel E. **EDPC: a novel high affinity ligand for the benzodiazepine site on rat GABA (A) receptors.** Neurosci Lett 1999;269(2):63-6.
Rat recombinant alpha1beta2gamma2 gamma-aminobutyric acid type A (GABAA) receptors were functionally expressed in Xenopus laevis oocytes and analyzed for the action of EDPC (Ethyl 3-(1,3-dithian-2-yl)-1H-pyrrolo[2,3-c]pyridine-5-carboxylate) using electrophysiological techniques. EDPC

inhibited GABA currents at low concentrations (IC50 approximately/= 2 nM). The inhibition by 100 nM EDPC could be reversed by 1 microM of the benzodiazepine antagonistflumazenil (Ro 15-1788), indicating a negative allosteric modulation via the benzodiazepine binding site. In line with this conclusion are radioactive ligand binding studies. EDPC inhibited the binding of 2 nM [3H] flunitrazepam to membranes from the cerebellum or the cortex with IC50 values of about 8 and 25 nM, respectively.

Tiedge M, Lortz S, Munday R, Lenzen S. **Protection against the co-operative toxicity of nitric oxide and oxygen free radicals by overexpression of antioxidant enzymes in bioengineered insulin-producing RINm5F cells.** Diabetologia 1999;42(7):849-55.
AIMS/HYPOTHESIS: The importance of different antioxidative enzymes for the defence of insulin-producing cells against the toxicity of nitric oxide (NO) was characterised in bioengineered RINm5F cells. METHODS: RINm5F insulin-producing cells stably overexpressing glutathione peroxidase (GPX), catalase (CAT) or Cu/Zn superoxide dismutase (SOD) were exposed to S-nitroso-N-acetyl-D,L-penicillamine (SNAP), sodium nitroprusside (SNP) and 3 morpholinosydnonimine (SIN-1), which generate both NO and reactive oxygen species, and to the polyamine/ NO, complex DETA/NO which generates NO alone. The viability of the cells was tested by the MTT assay. RESULTS: Overexpression of antioxidant enzymes provided significant protection against the toxicity of SNAP, SNP and SIN-1, with an individual specificity related to their chemical characteristics, but was without effect upon the toxicity of DETA/NO. Cells overexpressing GPX were well protected against SNP and SNAP, while CAT was most effective against SIN-1. SOD overexpression provided less protection against the toxicity of SNAP and SNP than overexpression of GPX but was more effective in protecting against SIN-1. Co-incubation of cells with NO donors and hydrogen peroxide or hypoxanthine and xanthine oxidase showed an overadditive synergism of toxicity. CONCLUSION/INTERPRETATION: The results emphasise the importance of a synergism between NO and reactive oxygen species for pancreatic beta-cell death. Such a synergism has also been observed after exposure of beta cells to cytokines. The component of the toxicity that is mediated by oxygen radicals can be suppressed effectively through overexpression of CAT, GPX or SOD or both.

Ueda A. **Adsorption of salivary proteins and serum proteins to titanium.** J Osaka Dent Univ 1998;32 (2):59-65.
Titanium (Ti) is very effective as a dental implant material owing to its low toxicity and high biocompatibility. I carried out in vitro experiments on the adsorption of salivary proteins and serum proteins to Ti to better understand how this material reacts in the oral cavity. I found that when Ti that had adsorbed serum proteins was exposed to salivary proteins, there was an exchange of the proteins on the Ti surface. The same thing happened when Ti that had adsorbed salivary proteins was exposed to serum proteins. These results suggest that both human salivary proteins and serum proteins adsorb to Ti with different affinity, and that protein already adsorbed to Ti might mask binding sites for other proteins.

Volkel W, Pahler A, Dekant W. **Gas chromatography-negative ion chemical ionization mass spectrometry as a powerful tool for the detection of mercapturic acids and DNA and protein adducts as biomarkers of exposure to halogenated olefins.** J Chromatogr A 1999;847(1-2):35-46.
The studies on metabolism of halogenated olefins presented here outline the advantages of modern mass

spectrometry. The perchloroethene (PER) metabolite N-acetyl-S-(trichlorovinyl)-L-cysteine (N-ac-TCVC) is an important biomarker for the glutathione dependent biotransformation of PER. In urine of rats and humans exposed to PER, N-ac-TCVC was quantified as methyl ester after BF3-MeOH derivatization by gas chromatography with chemical ionization and negative ion detection mass spectrometry (GC-NCI-MS). The detection limit was 10 fmol/microliter injected solution using [2H3]N-ac-TCVC methyl ester as the stable isotope internal standard. Cleavage of S-(trichlorovinyl)-L-cysteine by beta-lyase enzymes results in an electrophilic and highly reactive thioketene which reacts with nucleophilic groups in DNA and proteins. Protein adduct formation was shown in kidney mitochondria by identification of dichloroacetylated lysine after derivatization with 1,1,3,3-tetrafluoro-1,3-dichloroacetone by GC-NCI-MS. In addition, chlorothioketene was generated in organic solvents and reacted with cytosine to give N4-chlorothioacetyl cytosine. After derivatization with pentafluorobenzyl bromide this compound exhibited good gas chromatographic properties and was detectable with a limit of detection of 50 fmol/injected volume. The detection of chemically induced protein modifications in the target organ of toxic metabolite formation and the study of DNA modifications with chemically generated metabolites provide important information on organ toxicity and possible tumorigenicity of halogenated olefins.

Voskoboinik I, Strausak D, Greenough M, Brooks H, Petris M, Smith S, Mercer JF, Camakaris J. **Functional analysis of the N-terminal CXXC metal-binding motifs in the human menkes copper-transporting P-type ATPase expressed in cultured mammalian cells.** J Biol Chem 1999;274 (31):22008-12.
The Menkes protein (MNK) is a copper-transporting P-type ATPase, which has six highly conserved metal-binding sites, GMTCXXC, at the N terminus. The metal-binding sites may be involved in MNK trafficking and/or copper-translocating activity. In this study, we report the detailed functional analysis in mammalian cells of recombinant human MNK and its mutants with various metal-binding sites altered by site-directed mutagenesis. The results of the study, both in vitro and in vivo, provide evidence that the metal-binding sites of MNK are not essential for the ATP-dependent copper-translocating activity of MNK. Moreover, metal-binding site mutations, which resulted in a loss of ability of MNK to traffick to the plasma membrane, produced a copper hyperaccumulating phenotype. Using an in vitro vesicle assay, we demonstrated that the apparent K(m) and V(max) values for the wild type MNK and its mutants were not significantly different. The results of this study suggest that copper-translocating activity of MNK and its copper-induced relocalization to the plasma membrane represent a well coordinated copper homeostasis system. It is proposed that mutations in MNK which alter either its catalytic activity or/and ability to traffick can be the cause of Menkes disease.

Walden PD, Lefkowitz GK, Ittmann M, Lepor H, Monaco ME. **Mitogenic activation of human prostate-derived fibromuscular stromal cells by bradykinin.** Br J Pharmacol 1999;127(1):220-6.
Biologically active kinin peptides are released from precursor kininogens by kallikreins. Kinins act on kinin receptors to mediate diverse biological functions including smooth muscle contraction, inflammation, pain and mitogenicity. All components of the kallikrein-kinin system exist in human male genital secretions suggesting that these molecules participate in physiological and pathophysiological genitourinary function. The objective of this study was to assess the consequences of kinin action on prostate cells. Primary cultures of prostate secretory epithelial (PE) and prostate fibromuscular stromal

(PS) cells were established from human prostate tissue. Transcripts encoding both the human B1 and B2 bradykinin receptor subtypes were detected in human prostate transition-zone tissue and in cultured cells by RT-PCR. In receptor binding assays, the B1 subtype predominated on PE cell membranes and the B2 subtype predominated on PS cell membranes. In PS cells, but not in PE cells, BK induced significant inositol phosphate accumulation and [3H]-thymidine uptake. These responses were mediated through the B2 receptor subtype. The use of signal transduction inhibitors indicated that mitogenic activation by BK occurred through both protein kinase C (PKC) and protein tyrosine kinase dependent mechanisms. PMA (phorbol 12-myristate 13-acetate) produced maximal [3H]-thymidine uptake by PS cells, resulted in cell elongation and caused the alpha-actin fibres present in PS smooth muscle cells to became organized into parallel arrays along the length of the elongated cells. In summary, the prostate contains a functional kallikrein-kinin system, which could be significant in physiological and pathophysiological prostate function.

Wang HH, Lautt WW. **Evidence of nitric oxide, a flow-dependent factor, being a trigger of liver regeneration in rats.** Can J Physiol Pharmacol 1998;76(12):1072-9.
The hypothesis tested was that the hemodynamic consequence of partial hepatectomy (PHX) triggers the cascade of events that leads to liver regeneration. After PHX, all the portal flow must go through the remaining vascular bed, thus producing increased shear stress and release of nitric oxide (NO), which then initiates the next stages of the regeneration process. As an index of triggering of the regeneration cascade, we used an in vitro bioassay detecting the appearance of proliferating factors (PFs; various growth factors, cytokines, and hormones) in plasma 4 h after two-thirds PHX in rats. PF levels, assessed using proliferation of cultured hepatocytes, were elevated in two-thirds PHX rats, fully blocked by the NO synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME), and restored by L-arginine. L-NAME inhibited liver weight restoration at 48 h but resulted in high mortality. L-NAME lacked toxic effects in non-PHX rats. NO was directly antiproliferative on cultured cells, suggesting that the proliferative effect of NO in vivo was secondary to the activation of other proliferative stimuli. The data support the hypothesis that vascular shear stress induced release of NO following PHX serves as a primary trigger to initiate the regeneration process.

Wang X, Zhou A, Liu M, Yu H, Pang L, Zhu M, Wang L, Berg H. **Effects of ELF capacitively coupled weak electric fields on metabolism of 6B1 cells.** Bioelectrochem Bioenerget 1999;48(2):369-73.
BIOSIS COPYRIGHT: BIOL ABS. In this study, we adopted several methods of MTT colorimetry, DAPI fluorimetry and ELISA to study the effects of extremely low frequency (ELF) capacitively coupled electric fields (EFs) on the metabolism of 6B1 cells. The result shows that 50 mV cm-1 ELF EF (10-100 Hz) has no significant effect on proliferation, DNA synthesis and activity of succinate dehydrogenase of 6B1 cells, indicating that the effect of ELF (10-100 Hz) EF on the metabolism of 6B1 cells is not obvious. However, 50 mV cm-1, 5.

Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. **Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis.** Proc Natl Acad Sci U S A 1999;96(13):7563-8.
The beneficial actions of nonsteroid anti-inflammatory drugs (NSAID) can be associated with inhibition of cyclo-oxygenase (COX)-2 whereas their harmful side effects are associated with inhibition of COX-1.

Here we report data from two related assay systems, the human whole blood assay and a modified human whole blood assay (using human A549 cells as a source of COX-2). This assay we refer to as the William Harvey Modified Assay. Our aim was to make meaningful comparisons of both classical NSAIDs and newer COX-2-selective compounds. These comparisons of the actions of >40 NSAIDs and novel COX-2-selective agents, including celecoxib, rofecoxib and diisopropyl fluorophosphate, demonstrate a distribution of compound selectivities toward COX-1 that aligns with the risk of serious gastrointestinal complications. In conclusion, this full in vitro analysis of COX-1/2 selectivities in human tissues clearly supports the theory that inhibition of COX-1 underlies the gastrointestinal toxicity of NSAIDs in man.

Weintraub BD, Szkudlinski MW. **Development and in vitro characterization of human recombinant thyrotropin.** Thyroid 1999;9(5):447-50.
We have gained insight into the molecular mechanism of human thyrotropin (hTSH) action through cloning of the human TSHbeta subunit gene, development of recombinant TSH and novel analogues and chimeras produced by site-directed as well as cassette mutagenesis. A variety of loss of function mutations have shown several key domains in both the alpha- and beta-subunits that are important for high-affinity ligand interaction with the receptor. In contrast the specificity of receptor interaction was shown to be determined primarily by areas within the hTSH-beta "seat-belt" region. We have also designed various gain of function mutants (superagonists) using evolutionary considerations, homology modeling, and sequence comparisons within the cystine knot growth factor superfamily. Such superagonists resulted from increasing the positive charge by introduction of lysine or arginine residues or neutralization of negatively charged residues of the peripheral hairpin loops of each subunit in various combinations. Certain superagonists increased receptor binding, in vitro and in vivo bioactivity 100- to 1000-fold, more than that achieved previously for any other known protein ligand. In vivo metabolic clearance and biologic activity could be separately modulated by alteration of TSH carbohydrate structure including production of chimeras that added sites of O-glycosylation and/or covalently linked the alpha- and beta-subunits. These data suggest that electrostatic interactions resulting from net positive charge in TSH and net negative charge in its receptor play an important role in high-affinity TSH receptor binding and signal transduction. Insights gained from the design of such novel recombinant TSH analogues and chimeras should have many diagnostic and therapeutic applications. These include the design of improved in vitro assays for thyrotropic factors as well as the design of second generation recombinant TSH analogues for the detection and treatment of thyroid cancer.

Wiemels J, Smith MT. **Enhancement of myeloid cell growth by benzene metabolites via the production of active oxygen species.** Free Radic Res 1999;30(2):93-103.
In low concentrations, benzene and its metabolite hydroquinone are known to have diverse biological effects on cells, including the synergistic stimulation with GM-CSF of hematopoietic colony formation in vitro, stimulation of granulocytic differentiation in vitro and in vivo, and general suppression of hematopoiesis in vivo. These chemicals are also known to be active in the induction of active oxygen species. We used several assays to determine the effects of benzene metabolites (hydroquinone, benzenetriol, benzoquinone) and active oxygen species (xanthine/xanthine oxidase) on cell growth and cell cycle kinetics of the human myeloid cell line HL-60. HL-60 cells treated with these chemicals for 2 h in PBS showed increased growth over untreated controls in a subsequent 18h growth period in

complete media. Incorporation of 3H-thymidine was also increased proportionately by these treatments. Catalase treatment abrogated the increased cell growth of all chemicals, suggesting an oxidative mechanism for the effect of all treatments alike. Cell cycle kinetics assays showed that the growth increase was caused by an increased recruitment of cells from G0/G1 to S-phase for both hydroquinone and active oxygen, rather than a decrease in the length of the cell cycle. Benzene metabolite's enhancement of growth of myeloid cells through an active oxygen mechanism may be involved in a number of aspects of benzene toxicity, including enhanced granulocytic growth and differentiation, stimulation of GM-CSF-induced colony formation, apoptosis inhibition, and stimulation of progenitor cell mitogenesis in the bone marrow. These effects in sum may be involved in the benzene-induced "promotion" of a clonal cell population to the fully leukemic state.

Wiren K, Keenan E, Zhang X, Ramsey B, Orwoll E. **Homologous androgen receptor up-regulation in osteoblastic cells may be associated with enhanced functional androgen responsiveness.** Endocrinology 1999;140(7):3114-24.
Although androgens have myriad effects on the skeleton, the regulation of androgen action in bone is not well understood. Androgen receptors (ARs) are known to play an important role in mediating androgen action. We have examined the effects of androgens and other sex steroids on AR levels in osteoblastic cells in vitro using two clonal human cell lines, SaOS-2 and U-2 OS. AR protein levels were quantitated both by specific androgen binding studies and Western analyses, and AR messenger RNA was measured with RNase protection assays. Potential changes in AR functionality was assessed by reporter assays. Treatment of osteoblastic cells with the nonaromatizable androgen 5alpha-dihydrotestosterone (DHT) increased specific androgen binding 2-to 4-fold. Similar increases in AR protein levels were documented by Western analysis in both cell lines. The androgen-mediated increase in receptor levels was time and dose dependent as well as androgen specific. Steady-state AR messenger RNA levels were also increased by DHT. When AR concentrations in osteoblastic cells were elevated with exogenous receptor, there was an enhancement of DHT responsiveness, measured by increased trans-activation of an androgen-responsive promoter. Thus, androgen exposure increased androgen receptor protein levels and specific androgen binding in osteoblastic cells. Androgen action as measured by androgen-mediated transcriptional activation is enhanced in the presence of elevated AR levels. Consequently, these studies have revealed an additional means by which androgens may modulate skeletal metabolism.

Xue LL, Wang YH, Xie Y, Yao P, Wang WH, Qian W, Huang ZX, Wu J, Xia ZX. **Effect of mutation at valine 61 on the three-dimensional structure, stability, and redox potential of cytochrome b5.** Biochemistry 1999;38(37):11961-72.
To elucidate the role played by Val61 of cytochrome b(5), this residue of the tryptic fragment of bovine liver cytochrome b(5) was chosen for replacement with tyrosine (Val61Tyr), histidine (Val61His), glutamic acid (Val61Glu), and lysine (Val61Lys) by means of site-directed mutagenesis. The mutants Val61Tyr, Val61Glu, Val61His, and Val61Lys exhibit electronic spectra identical to that of the wild type, suggesting that mutation at Val61 did not affect the overall protein structure significantly. The redox potentials determined by differential pulse voltammetry were -10 (wild type), -25 (Val61Glu), -33 (Val61Tyr), 12 (Val61His), and 17 mV (Val61Lys) versus NHE. The thermal stabilities and urea-mediated denaturation of wild-type cytochrome b(5) and its mutants were in the following order: wild type > Val61Glu > Val61Tyr > Val61His > Val61Lys. The kinetics of denaturation of cytochrome b(5)

by urea was also analyzed. The first-order rate constants of heme transfer between cytochrome b(5) and apomyoglobin at 20 +/- 0.2 degrees C were 0.25 +/- 0.01 (wild type), 0.42 +/- 0.02 (Val61Tyr), 0.93 +/- 0.04 (Val61Glu), 2.88 +/- 0.01 (Val61His), and 3.88 +/- 0.02 h(-)(1) (Val61Lys). The crystal structure of Val61His was determined using the molecular replacement method and refined at 2.1 A resolution, showing that the imidazole side chain of His61 points away from the heme-binding pocket and extends into the solvent, the coordination distances from Fe to NE2 atoms of two axial ligands are approximately 0.6 A longer than the reported value, and the hydrogen bond network involving Val61, the heme propionates, and three water molecules no longer exists. We conclude that the conserved residue Val61 is located at one of the key positions, the "electrostatic potential" around the heme-exposed area and the hydrophobicity of the heme pocket are determinant factors modulating the redox potential of cytochrome b(5), and the hydrogen bond network around the exposed heme edge is also an important factor affecting the heme stability.

Yamaguchi R, Hirano T, Ootsuyama Y, Asami S, Tsurudome Y, Fukada S, Yamato H, Tsuda T, Tanaka I, Kasai H. **Increased 8-hydroxyguanine in DNA and its repair activity in hamster and rat lung after intratracheal instillation of crocidolite asbestos.** Jpn J Cancer Res 1999;90(5):505-9.
Asbestos and man-made-mineral fibers are known to increase one type of oxidative DNA damage, 8-hydroxyguanine (8-OH-(Gua), in vitro. In this study, we analyzed the 8-OH-Gua level in DNA and its repair activity after a single intratracheal instillation of fibers (crocidolite or glass) or saline to Syrian hamsters or Wistar rats. The 8-OH-Gua level was measured with a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system. The 8-OH-Gua repair enzyme activity was determined with an endonuclease nicking assay using a 32P-labeled or fluorescently labeled 22mer DNA that contains 8-OH-Gua at a specific position. A significant increase in the 8-OH-Gua level in the lung DNA was observed 1 day after the exposure to crocidolite, as compared to the saline control. The repair activity was increased significantly at 7 days. On the other hand, after exposure to glass fibers, little or no increase of these carcinogenicity indicators was detected. These assays of 8-OH-Gua and its repair activity in short-term animal experiments will be useful for evaluating the carcinogenicity of fibers. This is the first report of the increase of 8-OH-Gua and its repair activity in the animal lung after the instillation of asbestos fibers.

Yamamoto H, Soh JW, Monden T, Klein MG, Zhang LM, Shirin H, Arber N, Tomita N, Schieren I, Stein CA, et al. **Paradoxical increase in retinoblastoma protein in colorectal carcinomas may protect cells from apoptosis.** Clin Cancer Res 1999;5(7):1805-15.
The retinoblastoma (Rb) gene is inactivated in a variety of human cancers, but in colorectal carcinomas there is frequently increased expression of this gene. This is paradoxical in view of the known role of Rb as a tumor suppressor gene. In the present study, we compared the levels of expression of the Rb protein (pRb) in normal human colorectal mucosa, adenomatous polyps, and carcinomas by immunohistochemistry. In vitro studies were also done to examine the phenotypic effects of an antisense oligodeoxynucleotide (AS-Rb) targeted to Rb mRNA in the HCT116 colon carcinoma cell line that expresses a relatively high level of pRb. The incidence of pRb-positive cells was increased during multistage colorectal carcinogenesis. In vitro treatment of HCT116 cells with AS-Rb decreased the level of pRb by about 70% and also decreased the levels of the cyclin D1 protein and cyclin D1-associated kinase activity. AS-Rb inhibited growth of HCT116 cells and induced apoptosis. Reporter assays

indicated about a 17-fold increase in E2F activity. These findings suggest that the increased expression of pRb in colorectal carcinoma cells may provide a homeostatic mechanism that protects them from growth inhibition and apoptosis, perhaps by counterbalancing potentially toxic effects of excessive E2F activity.

Yamashita H, Avraham S, Jiang S, Dikic I, Avraham H. **The Csk homologous kinase associates with TrkA receptors and is involved in neurite outgrowth of PC12 cells.** J Biol Chem 1999;274 (21):15059-65.
Csk homologous kinase (CHK), a member of the Csk regulatory tyrosine kinase family, is expressed primarily in brain and hematopoietic cells. The role of CHK in the nervous system is as yet unknown. Using PC12 cells as a model system of neuronal cells, we show that CHK participates in signaling mediated by TrkA receptors. CHK was found to be associated with tyrosine-phosphorylated TrkA receptors in PC12 cells upon stimulation with NGF. Binding assays and far Western blotting analysis, using glutathione S-transferase fusion proteins containing the Src homology 2 (SH2) and SH3 domains of CHK, demonstrate that the SH2 domain of CHK binds directly to the tyrosine-phosphorylated TrkA receptors. Site-directed mutagenesis of TrkA cDNA, as well as phosphopeptide inhibition of the in vitro interaction of the CHK-SH2 domain or native CHK with TrkA receptors, indicated that the residue Tyr-785 on TrkA is required for its binding to the CHK-SH2 domain upon NGF stimulation. In addition, overexpression of CHK resulted in enhanced activation of the mitogen-activated protein kinase pathway upon NGF stimulation, and microinjection of anti-CHK antibodies, but not anti-Csk antibodies, inhibited neurite outgrowth of PC12 cells in response to NGF. Thus, CHK is a novel signaling molecule that participates in TrkA signaling, associates directly with TrkA receptors upon NGF stimulation, and is involved in neurite outgrowth of PC12 cells in response to NGF.

Yamori T, Matsunaga A, Sato S, Yamazaki K, Komi A, Ishizu K, Mita I, Edatsugi H, Matsuba Y, Takezawa K, et al. **Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an in vitro and in vivo human cancer cell line panel.** Cancer Res 1999;59(16):4042-9.
We synthesized a novel anticancer agent MS-247 (2-[[N-[1-methyl-2-[5-[N-[4-[N,N-bis(2-chloroethyl) amino] phenyl]] carbamoyl]-1H-benzimidazol-2-yl] pyrrol-4-yl] carbamoyl] ethyldimethylsulfonium di-p-toluenesulfonate) that has a netropsin-like moiety and an alkylating residue in the structure. We evaluated antitumor activity of MS-247 using a human cancer cell line panel coupled with a drug sensitivity database and subsequently using human cancer xenografts. The average MS-247 concentration required for 50% growth inhibition against a panel of 39 cell lines was 0.71 microM. The COMPARE analysis revealed that the differential growth inhibition pattern of MS-247 significantly correlated with those of camptothecin analogues and anthracyclins, indicating that MS-247 and the two drug groups might have similar modes of action. MS-247 exhibited remarkable antitumor activity against various xenografts. A single i.v. injection of MS-247 significantly inhibited the growth of all 17 xenografts tested, which included lung, colon, stomach, breast, and ovarian cancers. In many cases, MS-247 was more efficacious than cisplatin, Adriamycin, 5-fluorouracil, cyclophosphamide, VP-16, and vincristine and was almost comparable with paclitaxel and CPT-11; these are the most clinically promising drugs at present. MS-247 was noticeably more effective than paclitaxel (in HCT-15) and CPT-11 (in A549, HBC-4, and SK-OV-3). The toxicity of MS-247, indicated by body weight loss, was reversible within 10 days after administration. The MS-247 mode of action showed DNA binding

activity at the site where Hoechst 33342 bound, inhibited topoisomerases I and II (as expected by the COMPARE analysis) blocked the cell cycle at the G2-M phase, and induced apoptosis. These results indicate that MS-247 is a promising new anticancer drug candidate to be developed further toward clinical trials.

Yang C, Chen S. **Two organochlorine pesticides, toxaphene and chlordane, are antagonists for estrogen-related receptor alpha-1 orphan receptor.** Cancer Res 1999;59(18):4519-24.
Estrogen-related receptor (ERR) alpha-1 shares a high amino acid sequence homology with estrogen receptor alpha. Although estrogens are not ligands of ERR alpha-1, our recent results suggest that toxaphene and chlordane, two organochlorine pesticides with estrogen-like activity, behave as antagonists for this orphan nuclear receptor. The two compounds increased ERR alpha-1-mediated expression of the reporter enzyme beta-galactosidase in a yeast-based assay. The screen was developed by expressing the hERR alpha-1-yeast Gal 4 activation domain fusion protein in yeast cells carrying the beta-galactosidase reporter plasmid, which contains an ERR alpha-1-binding element. In transfection experiments using mammalian cell lines, such as the SK-BR-3 breast cancer cell line, the compounds were found to have an antagonist activity against ERR alpha-1-mediated expression of the reporter chloramphenicol acetyltransferase. In contrast to the findings with ERR alpha-1, the two compounds were found to slightly induce the estrogen receptor a-mediated expression of chloramphenicol acetyltransferase in SK-BR-3 cells. In a ligand-independent manner, the ERR alpha-1 activity in SK-BR-3 cells was induced 3-fold by cotransfection with the GRIP1 coactivator expression plasmid. Toxaphene was found to be capable of suppressing the GRIP1 coactivator-induced ERR alpha-1 activity in SK-BR-3 cells. In addition, a stable ERR alpha-1 expressing HepG2 hepatoma cell line was generated, and the aromatase activity in the transfected cell line was found to be twice that in the untransfected cell line. The enzyme aromatase converts androgens to estrogens, and aromatase expression in HepG2 cells is regulated in part by an ERR alpha-1-modulating promoter. A 24-h incubation of an ERR alpha-1-transfected HepG2 cell line with 10 microM toxaphene reduced its aromatase activity to the level in the untransfected cell line. Because toxaphene is not an inhibitor of aromatase, it is thought that the decrease of the aromatase activity in ERR alpha-1 transfected HepG2 cells following toxaphene treatment resulted from a suppression of the aromatase expression by toxaphene acting as the antagonist of ERR alpha-1. Toxaphene and chlordane are among the 12 persistent organic pollutants identified by the United Nations Environment Programme as requiring urgent attention. Their antagonistic effects on ERR alpha-1 should not be overlooked.

Yu D. **A physiologically based pharmacokinetic model of inorganic arsenic.** Regul Toxicol Pharmacol 1999;29(2 Pt 1):128-41.
This study presents a physiologically based pharmacokinetic model of inorganic arsenic disposition in humans. The model focuses on short-term exposures by the oral route. The model considers the four circulating species (AsIII, AsV, and two metabolites, i.e., monomethylarsenic (MMA) and dimethylarsenic (DMA)) in tissue groups. The model also provides for the reduction of AsV to AsIII via chemical reaction with tissue glutathione and the subsequent transformation of AsIII into two metabolites (MMA and DMA) based on the experimental observations. Effort on the development of the model is directed toward the prediction of the kinetic behavior of inorganic arsenic in the body, following environmental exposure at ambient water concentrations, including tissue and blood

concentrations, and especially urinary excretion of arsenic and its methylated metabolites. While it is difficult to estimate some of parameters used in the model at this time, the current model assumptions and predictions seem to be consistent with the experimental observations found in the literature. Therefore, the current model, when more fully developed, is expected to provide insight into the behavior of inorganic arsenic and its methylated metabolites within the body and may help increase the understanding of risk assessment issues associated with inorganic arsenic in drinking water. Copyright 1999 Academic Press.

Yuan B, Sun Z. **[Transformation of rat hepatocytes in an in vitro primary culture by aflatoxin B1]**. Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao 1997;19(1):6-10. (Chi)
Aflatoxin B1 (AFB1) is one of the major causative factors of hepatocellular carcinoma. In this study, the combined effects of AFB1 activated by human cytochrome p450 IA2 and c-myc in transformation of rat hepatocytes were investigated in an in vitro primary culture system. The expression vectors, Xm-6/c-myc was first constructed and their expression possibilities were examined in Alexander cells by immunocytochemistry. Then both c-myc and human cytochrome p450 IA2 expression vectors were sequentially transfected into newborn rat liver cells in serum-free primary culture. Results showed that p450 IA2 could activate AFB1 at concentrations as low as 5 ng/ml, and the activated AFB1 coupled with exogenous c-myc could induce rat hepatocytes to survive and grow beyond two-month limit in primary culture. During long-term in vitro culturing including four-month in crisis, one of the randomly selected transformed hepatocytes with the growth advantage became immortalized. Immunocytochemical assays for CK-18 and rat albumin plus observed electron microscopic features clearly confirmed these cells derived from epithelial hepatocytes. Further characterization showed that the process of immortalization was associated with chromosomal abnormalities and elevated expression of TGF alpha.

Yukawa E. **Population-based investigations of drug relative clearance using nonlinear mixed-effect modelling from information generated during the routine clinical care of patients.** J Clin Pharm Ther 1999;24(2):103-13.
Interpatient variability in drug disposition and response is a therapeutic premise, and thus evaluation and management of such variability are the basis for individualized pharmacotherapy. If the mathematical approach to determining drug doses were accurate and practical, the use of calculated doses could reduce the potential for toxicity and decrease the need for repetitious drug assays. The major strength of the population pharmacokinetics approach is that useful information can be extracted from sparse data collected during routine clinical care. Population pharmacokinetics can be defined as the study of the variability in serum drug concentrations between individuals when standard dosage regimens are administered. An approach to population pharmacokinetic data analysis has been implemented in the Nonlinear Mixed Effects Model (NONMEM) computer program. This report shows the feasibility of using a simple pharmacokinetic screen approach to estimate the population mean relative drug clearance and detecting drug-drug interaction by use of NONMEM. In clinical application of multiple trough screen or multiple peak screen, the variability of drug relative clearance within the population is assessed and a mathematical relationship between drug relative clearance and individual patient characteristics, such as age, body weight, gender, disease state or drug interaction with concomitant drug is derived. In this report I describe this approach and its application using several examples previously

reported by us and others.

Zager RA. **Calcitriol directly sensitizes renal tubular cells to ATP-depletion- and iron-mediated attack.** Am J Pathol 1999;154(6):1899-909.
Vitamin Ds have been reported to have diverse effects on cell homeostasis, leading to suggestions that they have therapeutic applications extending beyond their traditional actions on the Ca2+/parathyroid/bone axis. As some of these potential indications carry an inherent risk of acute renal failure (ARF; eg, cancer chemotherapy and organ transplantation), the goal of this study was to assess whether vitamin Ds directly affect renal tubule injury responses. Cultured human proximal tubular (HK-2) cells were exposed to physiological or pharmacological doses of either calcitriol (D3) or a synthetic vitamin D2 analogue (19-nor) for 3 to 48 hours. Their impact on cell integrity (percent lactate dehydrogenase (LDH) release and tetrazolium dye MTT uptake) under basal conditions and during superimposed injuries (ATP depletion/Ca2+ ionophore or iron-mediated oxidant stress) were determined. As vitamin Ds can be anti-proliferative, cell outgrowth ([3H]thymidine uptake and crystal violet staining) was also tested. Finally, the action of D3 on in vivo ARF (glycerol-induced myoglobinuria) and isolated proximal tubule injury responses were assessed. D3 induced a rapid, dose-dependent increase in HK-2 susceptibility to both ATP-depletion/Ca2+-ionophore- and Fe-mediated attack without independently affecting cell integrity or proliferative responses. In contrast, D2 negatively affected only Fe toxicity and only after relatively prolonged exposure (48 hours). D3 dramatically potentiated in vivo ARF (two- to threefold increase in azotemia), suggesting potential in vivo relevance of the above HK-2 cell results. Proximal tubules, isolated from these glycerol-exposed mice, suggested that D3 can worsen tubule injury despite a parodoxic suppression of H2O2 production. In contrast, D3 had a mild negative impact on cellular energetics (depressed ATP/ADP ratios), and it accentuated plasma membrane phospholipid breakdown. The latter was observed in both glycerol-treated and control tubules, suggesting a primary role in the injury- potentiation effect of D3. Vitamins D(s) may directly, and differentially, increase proximal tubule cell susceptibility to superimposed attack. This property should be considered as new uses for these agents are defined.

Zaulyanov LL, Green PS, Simpkins JW. **Glutamate receptor requirement for neuronal death from anoxia-reoxygenation: an in vitro model for assessment of the neuroprotective effects of estrogens.** Cell Mol Neurobiol 1999;19(6):705-18.
1. Previous studies demonstrated that estrogens, specifically 17 beta-estradiol, the potent, naturally occurring estrogen, are neuroprotective in a variety of models including glutamate toxicity. The aim of the present study is twofold: (1) to assess the requirement for glutamate receptors in neuronal cell death associated with anoxia-reoxygenation in three cell types, SK-N-SH and HT-22 neuronal cell lines and primary rat cortical neuronal cultures, and (2) to evaluate the neuroprotective activity of both 17 beta-estradiol and its weaker isomer, 17 alpha-estradiol, in both anoxia-reoxygenation and glutamate toxicity. 2. SK-N-SH and HT-22 cell lines, both of which lack NMDA receptors as assessed by MK-801 binding assays, were resistant to both anoxia-reoxygenation and glutamate-induced cell death. In contrast, primary rat cortical neurons, which exhibit both NMDA and AMPA receptors, were sensitive to brief periods of exposure to anoxia-reoxygenation or glutamate. As such, there appears to be an obligatory requirement for NMDA and/or AMPA receptors in neuronal cell death resulting from brief periods of anoxia followed by reoxygenation. 3. Using primary rat cortical neuronal cultures, we evaluated the

neuroprotective activity of 17 beta-estradiol (1.3 or 133 nM) and 17 alpha-estradiol (133 nM) in both anoxia-reoxygenation and excitotoxicity models of cell death. We found that the 133 nM but not the 1.3 nM dose of the potent estrogen, 17 beta-estradiol, protected 58.0, 57.5, and 85.3% of the primary rat cortical neurons from anoxia-reoxygenation, glutamate, or AMPA toxicity, respectively, and the 133 nM dose of the weak estrogen, 17 alpha-estradiol, protected 74.6, 81.7, and 85.8% of cells from anoxia-reoxygenation, glutamate, or AMPA toxicity, respectively. These data demonstrate that pretreatment with estrogens can attenuate glutamate excitotoxicity and that this protection is independent of the ability of the steroid to bind the estrogen receptor.

Zhao F, Mayura K, Kocurek N, Edwards JF, Kubena LF, Safe SH, Phillips TD. **Inhibition of 3,3',4,4',5-pentachlorobiphenyl-induced chicken embryotoxicity by 2,2',4,4',5,5'-hexachlorobiphenyl.** Fundam Appl Toxicol 1997 Jan;35(1):1-8.
3,3',4,4',5-Pentachlorobiphenyl (pentaCB) caused a dose-dependent induction of chicken embryolethality, malformations, edema, and liver lesions at doses ranging from 0.5 to 12.0 microg/kg. In contrast, no embryotoxicity was observed after treatment with 10, 25, or 50 mg/kg 2,2',4,4',5,5'-hexaCB. In eggs cotreated with 2.0 microg/kg, 3,3',4,4',5-pentaCB plus 10, 25, or 50 mg/kg 2,2',4,4',5,5'-hexaCB, there was significant protection from 3,3',4,4',5-pentaCB-induced embryo malformations, edema, and liver lesions, whereas no inhibition of embryolethality was observed. These results further extend the response-specific nonadditive interactions of binary mixtures of polychlorinated biphenyls (PCBs) and should be considered in the development of approaches for hazard assessment of PCB mixtures and related compounds.

Zhao HW, Lu CJ, Yu RJ, Hou XM. **An increase in hyaluronan by lung fibroblasts: a biomarker for intensity and activity of interstitial pulmonary fibrosis?** Respirology 1999;4(2):131-8.
The purpose of the present study was to clarify the roles of hyaluronan (HA) production of lung fibroblasts in the pathogenesis of pulmonary fibrosis. Quantitative and comparative assessments of the HA levels in bronchoalveolar lavage fluid (BALF) and lung fibroblast-conditioned media (F-CM) were made at various stages during the development of bleomycin-induced pulmonary fibrosis in rats. In bleomycin-treated animals, the HA levels in F-CM increased significantly ($P < 0.01$) on day 1 after bleomycin treatment, peaked on day 3, and then gradually declined and returned to control values on days 14-28. The HA concentrations of BALF in the bleomycin group were significantly increased ($P < 0.01$) on day 3, were maximal on day 7, and thereafter gradually decreased, remaining significantly above normal values ($P < 0.01$) on day 14, but returning to control values by day 28. In the bleomycin group, the HA levels both in BALF and in F-CM were significantly correlated with the cell components in BALF and there was a significant correlation between the HA concentration in BALF and in the F-CM. Lung fibroblasts were activated and produced increased HA which resulted in excessive accumulation of HA in the lung in the early stage of pulmonary fibrosis; the increased HA synthesis of lung fibroblasts and enhanced HA concentrations of BALF might reflect the intensity of alveolitis and the disease activity.

PULMONARY TOXICITY

Bellocq A, Azoulay E, Marullo S, Flahault A, Fouqueray B, Philippe C, Cadranel J, Baud L. **Reactive**

oxygen and nitrogen intermediates increase transforming growth factor-beta1 release from human epithelial alveolar cells through two different mechanisms. Am J Respir Cell Mol Biol 1999;21(1):128-36.
Transforming growth factor (TGF)-beta1 is a growth factor involved in the mechanisms of lung repair and fibrosis that follow inflammatory processes. We sought to examine the link between the generation of reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI) by inflammatory cells and the expression of TGF-beta1 by alveolar epithelial cells. Exposure of the A549 lung epithelial cell line to either an ROI generating system (xanthine and xanthine oxidase) or an RNI donor (S-nitroso-N-acetyl-penicillamine [SNAP]) promoted a time- and dose-dependent increase in TGF-beta1 release, as measured by a specific enzyme-linked immunosorbent assay. At the peak, the levels of TGF-beta1 were twice the control values. The induction of TGF-beta1 release by ROI was blunted by catalase and unaffected by superoxide dismutase, indicating the involvement of hydrogen peroxide. The response was also blunted by 5, 6-dichloro-1-beta-D-ribofuranosyl benzimidazole (DRB), a specific RNA polymerase II inhibitor, and accompanied by a corresponding increase in TGF-beta1 messenger RNA, as measured by quantitative/competitive reverse transcription polymerase chain reaction, suggesting the involvement of transcriptional mechanisms and possibly other downstream mechanisms. In contrast, RNI-induced TGF-beta1 release was unaffected by DRB and blunted by the protein synthesis inhibitor cycloheximide, suggesting the involvement of translational and post-translational mechanisms. This response required cyclic guanosine monophosphate (cGMP)- mediated processes because (1) immunoreactive cGMP accumulated in the culture medium of SNAP-treated cells; (2) SNAP-induced TGF-beta1 release was blunted by KT 5823, an inhibitor of cGMP-dependent protein kinase; and (3) similar increase in TGF-beta1 release was obtained by cell exposure to membrane-permeable dibutyryl-cGMP or to atrial natriuretic factor, a known agonist of particulate guanylate cyclase. These data suggest that in vitro exposure of human alveolar epithelial cells to ROI and RNI enhances TGF-beta1 release through different mechanisms. In vivo, this control may constitute a molecular link between inflammatory and fibrotic processes.

Dahlin KL, Bohlin K, Strindlund J, Ryrfeldt A, Cotgreave IA. **Amitriptyline-induced loss of tight junction integrity in a human endothelial--smooth muscle cell bi-layer model.** Toxicol 1999;136 (1):1-13.
Tricyclic antidepressants can, when taken in overdose, cause serious pulmonary failure such as the adult respiratory distress syndrome (ARDS). In this study we have examined the effects of some tricyclic antidepressants (amitriptyline, imipramine, nortriptyline and desipramine) on the viability and morphology of human endothelial and smooth muscle cells derived from umbilical cord. Effects of amitriptyline on endothelial cell fluidity, as well as permeability changes to an endothelial-smooth muscle cell bi-layer, were also studied. The tricyclic antidepressants induced acute, sub-lethal toxicity in both cell types above 100 microM as assessed by the MTT reduction assay. Morphological changes were also observed at these concentrations. Such changes were, however, absent at 33 microM and below. Amitriptyline did, however, cause a concentration-dependent fall in the electrical resistance of an endothelial-smooth muscle cell bi-layer, with significant effects already evident at 33 microM. All of these observed effects were fairly rapid and appeared within 5-15 min of exposure. The rapidity of these permeabilisation effects suggests potential membrane perturbations, since tricyclic antidepressants are lipophilic molecules with affinity for cell membranes. However, fluorescence anisotropy measurements

showed no significant difference in membrane fluidity between amitriptyline-treated and control endothelial cells. Collectively, these data point to specific mechanisms of action of amitriptyline, and probably also the other tricyclic antidepressants studied, on endothelial permeability, which is a hallmark of ARDS. The data suggest that increased endothelial permeability could be due to impaired tight junction function.

Marthan R. **[Cellular pathophysiologic effects of various air pollutants on the airways]**. Bull Acad Nat Med 1999;183(2):345-55, Discussion 355-6. (Fre)
Both epidemiological and experimental data indicate that major health consequences of urban air pollution i.e., respiratory symptoms are related, at least in part, to an alteration in the cellular processes implicated in bronchial responsiveness. In the Laboratory, we have set up techniques which enable to pre-expose in vitro human isolated bronchi to some air pollutants. Using these techniques, we have examined the dosimetric relationships and the cellular mechanisms of bronchial responsiveness altered by nitrogen dioxide (NO2), ozone (O3) or aldehydes such as acrolein. The combined effects of gas pollutants and passive immunological sensitization on bronchial hyperresponsiveness have also been studied. As a general rule, experimental protocols consisted in a comparison of the responsiveness of human bronchial tissues (obtained at thoracotomy from patients undergoing resection for pulmonary carcinoma) following ex vivo exposure to air pollutants with that of paired tissues unexposed to pollutants which acted as temporal controls. We have observed that the responsiveness of human isolated bronchi is increased following pre-exposure to NO2 or O3 or to acrolein for 15 to 30 min, at concentrations in the range of ppm and microM, respectively. A common target for the action of these pollutants on airway smooth muscle reactivity has been identified i.e., the release of intracellular stored calcium ions. Direct measurements of cytosolic calcium concentration in isolated airway smooth muscle cells exposed to pollutants have confirmed this hypothesis. Finally, we have obtained results indicating that passive sensitization and exposure to pollutants act in a additional manner on human bronchial smooth muscle reactivity in response to both specific antigen and non specific agonists. Collectively, these experimental in vitro results enable (i) to establish dosimetric relationships, (ii) to examine the cellular mechanisms and (iii) to identify populations at risk for various gas pollutants.

Monteil C, Le Prieur E, Buisson S, Morin JP, Guerbet M, Jouany JM. **Acrolein toxicity: comparative in vitro study with lung slices and pneumocytes type II cell line from rats.** Toxicology;133(2-3):129-38.
BIOSIS COPYRIGHT: BIOL ABS. Toxicological effects of acrolein have been studied in precision-cut rat lung slices and in L2 cells, a rat pneumocyte II cell line. These two models were cultured for 24 h with or without acrolein (0-100 muM in L2 cells; 0-200 muM in lung slices). Treatment with this pneumotoxicant produced a concentration dependent decrease in intracellular ATP levels. Acrolein concentrations higher than 50 muM induced ATP decrease in slices, while this decrease occurred from 10 muM acrolein in L2 cells. Detox vities as well as GSH levels were quickly decreased. In precision-cut rat lung slices, the induction of the glutathione pathway was less clear-cut. A bell-shaped dose response curve was observed with a maximum for 5 muM acrolein for GST and GRED activities. These differences between acrolein toxic ranges could be explained by the presence of an active detoxification pathway in slices compared to its relative lack in L2 cells.

Robledo RF, Barber DS, Witten ML. **Modulation of bronchial epithelial cell barrier function by in**

vitro jet propulsion fuel 8 exposure. Toxicol Sci 1999;51(1):119-25.
The loss of epithelial barrier integrity in bronchial and bronchiolar airways may be an initiating factor in the observed onset of toxicant-induced lung injuries. Acute 1-h inhalation exposures to aerosolized jet propulsion fuel 8 (JP-8) have been shown to induce cellular and morphological indications of pulmonary toxicity that was associated with increased respiratory permeability to 99mTc-DTPA. To address the hypothesis that JP-8 jet fuel-induced lung injury is initiated through a disruption in the airway epithelial barrier function, paracellular mannitol flux of BEAS-2B human bronchial epithelial cells was measured. Incubation of confluent cell cultures with non-cytotoxic concentrations of JP-8 or n-tetradecane (C14), a primary constituent of JP-8, for a 1-h exposure period resulted in dose-dependent increases of paracellular flux. Following exposures of 0.17, 0.33, 0.50, or 0.67 mg/ml, mannitol flux increased above vehicle controls by 10, 14, 29, and 52%, respectively, during a 2-h incubation period immediately after each JP-8 exposure. C14 caused greater mannitol flux increases of 37, 42, 63, and 78%, respectively, following identical exposure conditions. The effect on transepithelial mannitol flux reached a maximum at 12 h and spontaneously reversed to control values over a 48-h recovery period, for both JP-8 and C14 exposure. These data indicate that non-cytotoxic exposures to JP-8 or C14 exert a noxious effect on bronchial epithelial barrier function that may preclude pathological lung injury.

Sabaitis CP, Leong BK, Rop DA, Aaron CS. **Validation of intratracheal instillation as an alternative for aerosol inhalation toxicity testing.** J Appl Toxicol 1999;19(2):133-40.
In collecting inhalation toxicity data for the evaluation of the health hazard from occupational exposure to the aerosols of a drug or a chemical, the determination of the inhaled dose in relation to the animal response is most desirable. Intratracheal administration is most likely to deliver an exact dose of a compound to the lungs of an experimental animal. In a series of tests, microliter (microl) quantities of a solution or a suspension of a test material were nebulized into the trachea of an anesthetized rat using an intratracheal fast instillation (ITFI) method. The dose-response in terms of the minimal effective dose (MED) and the median lethal dose (LD50) were determined. The ITFI dose-response for four drugs, five chemicals or chemical intermediates and four pesticides were compared with those obtained via inhalation (IH) and ingestion (p.o.). In addition, the dose-responses of the four pesticides were compared with two additional parameters, intranasal instillation (IN) and intravenous injection (i.v.). The MED end-points for studies via the respiratory administration route were no pharmacotoxic signs other than transient respiratory rales and/or dyspnea and no gross lesions, whereas those for the intranasal, oral and the intravenous administration routes were transient and slight body weight loss and no pharmacotoxic signs and/or gross lesions. The MED ratios between ITFI, IH and p.o. were 1 : 9.3+/-6.5 : 201.4+/-133.3, respectively, for the drugs, chemicals and chemical intermediates. The MED ratios for ITFI, IH, IN, i.v. and p.o. for the four pesticides were 1 : 2.2+/-1.4 : 2.1+/-1.3 : 1.1+/-0.7 : 1.4+/-0.9. The MED ratios for the two categories of test materials were fairly consistent between different routes of administration. Thus, the ITFI dose can be used for extrapolating the IH dose. The simplicity of the ITFI procedure and its requirement of only microliters of a compound to generate a meaningful and reliable dose-response suggests that ITFI may be an alternative method for acute inhalation toxicity evaluation of materials that may present inhalation hazards from liquid or solid aerosols.

Wang Q, Fan J, Zhao X. **[Damage effects of asbestos and cigarette smoke solution on human embryo lung cell DNA]**. Chung Hua Yu Fang I Hsueh Tsa Chih 1998;32(1):31-3. (Chi)

OBJECTIVE: To study the combined damage effects of asbestos and cigarette solution on human embryo lung cell (HEL) DNA. METHODS: Unscheduled DNA synthesis (UDS) assay was used. Repair and synthesis on DNA in HEL treated with asbestos and/or cigarette smoke was studied. RESULTS: It showed that UDS in HEL could be induced by exposure to asbestos or cigarette smoke only with a significant dose-response relationship, and the amounts of [3H]-TdR incorporation in cells treated with combination of asbestos and cigarette smoke was significantly higher than their sum in cells treated with asbestos or cigarette only. CONCLUSION: There is a synergistic damage effect of asbestos and cigarette on DNA of HEL. In addition, dimethyl sulfoxide (DMSO), a scavenger of .OH, can partly inhibit [3H]-TdR incorporation caused by asbestos. And .OH plays certain role in damage to DNA of cells caused by asbestos.

Westmoreland C, Walker T, Matthews J, Murdock J. **Preliminary investigations into the use of a human bronchial cell line (16HBE14o-) to screen for respiratory toxins in vitro.** Toxicol In Vitro 1999;13(4-5):761-4.
BIOSIS COPYRIGHT: BIOL ABS. A transformed epithelial cell line derived from normal human bronchial epithelium (16HBE14o- cells) was used to assess the in vitro toxicity of six compounds. The compounds were sodium chloride and titanium dioxide (reference compounds) and sodium carbonate and silica (respiratory toxins). In addition, two compounds (compounds A and B) were tested which have been shown to induce respiratory toxicity in the rat during preclinical safety assessment. Confluent monolayers of 16HBE14o- cells were tre dpoints. With the exception of silica, all irritant compounds caused concentration-related cytotoxicity in 16HBE14o- cells. For each compound, when the three toxicity endpoints were compared, similar IC50 values were obtained irrespective of the endpoint used. These initial results indicate that 16HBE14o- cells may be a suitable cell line for future use in development of in vitro assays for respiratory toxicity.

Zhou J, Zhou L, Yang J. **[Studies on effects of cytokine released from alveolar macrophage induced by mineral dust on lung fibroblast]**. Chung Hua Yu Fang I Hsueh Tsa Chih 1998;32(6):336-9. (Chi)
OBJECTIVE: To understand the role of cytokine released from alveolar macrophage (AM) in lung fibrosis caused by mineral dust. METHODS: Rabbit's AM obtained by lavage was cultured with mineral dust in vitro. Activities of tumor necrosis factor (TNF) and interleukin 6 (IL-6) in its supernatant were determined with isotope labelling method and MTT colorimetry, respectively. Human fetal lung fibroblast WI-138 was cultured with this supernatant. Proliferation of fibroblast and synthesis of collagen were examined by 3H-thymidine (3H-TdR) and 14C-proline (14C-Pro) incorporation and its total hydroxyproline (HOP) level was analyzed by chloramine-T method. RESULTS: Proliferation of lung fibroblast and synthesis of collagen could be enhanced by the supernatant containing AM induced by quartz, asbestos and uranium mineral dust, with 3H-TdR incorporated counts per minute (cpm) of 6,584, 3,848 and 6,893 in the group of 100 micrograms 3H-TdR and 14C-Pro incorporated 27,952, 13,416 and 18,538 in the group of 200 micrograms respectively, which were significantly higher than those in the control group. Total HOP levels in the culture media for lung fibroblast enhanced by AM supernatant were 22.41, 24.00 and 21.39 micrograms/ml, respectively, and was significantly higher than that in the control group (12.91 micrograms/ml). Release of TNF and IL-6 could be stimulated by mineral dust, such as quartz, asbestos and uranium mineral dust, and their activities were significantly higher than those in the control group, with those of 1,396, 1,198 and 852 U/ml in TNF group and 1,336,

1511 and 1,335 U/ml in IL-6 group, respectively. Proliferation of lung fibroblast and synthesis of collagen could be inhibited by antibody against TNF and interferon-r (gamma), and the effect of the latter was weaker than that of the former on inhibition of fibroblast proliferation and the effect on collagen synthesis was just in the opposite direction. CONCLUSION: Lung fibrosis caused by mineral dust correlated with abnormal expression of TNF and IL-6. Antibody against TNF and gamma interferon could antagonize the effect of NTF and IL-6.

Zhou L, Zhou J, Yang J. **Effects of cytokines induced by mineral dust on lung fibroblasts in vitro.** J Occup Health 1999;41(3):144-8.
BIOSIS COPYRIGHT: BIOL ABS. Rabbit's alveolar macrophages (AM) obtained by lavage were cultured with three mineral dusts (quartz, asbestos fibre and uranium dust) in vitro. The activity of tumor necrosis factor (TNF) and lung fibroblast (LF) proliferation were measured by 3H-thymidine (3H-TdR) incorporation, the collagen synthesis in LF by 14C-proline (14C-Pro), interleukin-6 (IL-6) activity in the supernatant of AM by 3-(4,5-dimethyiazo-2-yl)-2,5-diphenyltetrazolium bromide; thiazoylblue (MTT) colorimetry, and the total h ls of IL-6 were 1336U/m/, 1511 U/m/ and 1335 U/m/, which were significantly higher than those in the TiO2 control. The LF proliferation and collagen synthesis can be increased by the supernatant of the AM treated with the three minerl dusts. 3H-TdR incorporations were 22320 dpm, 12547 dpm and 15048 dpm (at a 1:2 dilution of AM supernatant), which was significantly higher than in the TiO2 and Hank's control ($P<0.01$). 14C-Pro incorporations were 34001 dpm, 16319 dpm and 22550 dpm (at a dust concen rferon-gamma can inhibit the proliferation of the LF and decrease collagen synthesis.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Amishiro N, Okamoto A, Murakata C, Tamaoki T, Okabe M, Saito H. **Synthesis and antitumor activity of duocarmycin derivatives: modification of segment-A of A-ring pyrrole compounds.** J Med Chem 1999;42(15):2946-60.
A series of 3-substituted A-ring pyrrole compounds of duocarmycin were synthesized and evaluated for in vitro anticellular activity against HeLa S(3) cells and in vivo antitumor activity against murine sarcoma 180 in mice. These compounds were evaluated on the peripheral blood toxicity and delayed lethal toxicity. Further, to expand our investigation of their peripheral blood toxicity, the toxicity to bone marrow cells (CFU-GM, CFU-Meg) was investigated. Among 3-substituted A-ring pyrrole compounds of duocarmycin bearing a 5',6',7'-trimethoxy-2'-indolecarboxyl group as segment-B (Seg-B), several analogues showed remarkably potent antitumor activity with low peripheral blood toxicity. The 3-formyl compound 12h, one of such analogues, showed stronger antitumor activity with lower toxicity to bone marrow cells compared to DU-86 (2a), an active metabolite of KW-2189 (2b). However, compound 12h caused delayed death. On the other hand, the 3-bromo compound 15f, one of the 3-substituted A-ring pyrrole derivatives bearing a 4'-methoxycinnamoyl group as Seg-B, showed the most potent antitumor activity among the 4'-methoxycinnamate analogues with low toxicity to bone marrow cells. Furthermore, compound 15f did not cause delayed death similarly to 2d. These results would indicate the importance of the C-3 substituents of A-ring pyrrole duocarmycin derivatives for exhibiting antitumor activity and decreasing toxicity.

Baraldi PG, Cozzi P, Geroni C, Mongelli N, Romagnoli R, Spalluto G. **Novel benzoyl nitrogen mustard derivatives of pyrazole analogues of distamycin A: synthesis and antileukemic activity.** Bioorg Med Chem 1999;7(2):251-62.
The design and synthesis of novel benzoic acid mustard (BAM) derivatives of distamycin A bearing one or more pyrazole rings replacing the pyrrole rings of the latter are described. In vitro and in vivo activities against L1210 leukemia are reported and discussed. Some of these compounds show an activity profile comparable to tallimustine 1. All the compounds bearing the pyrazole ring close to the BAM moiety show reduced cytotoxicity in comparison to derivatives characterized by the BAM linked to a pyrrole: the same effect has not been observed when occurring at the amidine terminus of the oligopeptidic frame.

Briens F, Bureau R, Rault S. **Applicability of CATALYST in ecotoxicology, a new promising tool for 3D-QSAR: study of chlorophenols.** Ecotoxicol Environ Saf 1999;43(3):241-51.
With the aim of applying recent quantitative structure-activity relationship (QSAR) descriptors coming from the pharmaceutical sciences to the modelization of ecotoxicity data, this work deals with the establishment of 3D-QSAR on chlorophenols by using for the first time in this field the recent CATALYST software. The training set has been intentionally chosen simply because of its homogeneous character and the abundance of reliable experimental values. Among 69 data sets collected in the literature on these chemicals, 9 were selected to have fulfilled CATALYST simulation conditions. The principle of CATALYST is briefly described. The different investigations have led to nine 3D-QSAR called "hypotheses," meeting the criteria of high statistical significance attested by costs and correlation coefficient values. The ecotoxicity values calculated from the 9 models established in this study. Analysis of the graphical representations of hypotheses led to hypotheses about the toxicity mechanisms. A comparison is finally made between the CATALYST models and those previously derived by other modeling methods. Copyright 1999 Academic Press.

Chao Q, Deng L, Shih H, Leoni LM, Genini D, Carson DA, Cottam HB. **Substituted isoquinolines and quinazolines as potential antiinflammatory agents. Synthesis and biological evaluation of inhibitors of tumor necrosis factor alpha.** J Med Chem 1999;42(19):3860-73.
A series of isoquinolin-1-ones and quinazolin-4-ones and related derivatives were prepared and evaluated for their ability to inhibit tumor necrosis factor alpha (TNFalpha) production in human peripheral blood monocytes stimulated with bacterial lipopolysaccharide (LPS). In an effort to optimize the TNFalpha inhibitory activity, a homologous series of N-alkanoic acid esters was prepared. Several electrophilic and nucleophilic substitutions were also carried out. Alkanoic acid esters of four carbons were found to be optimum for activity in both the isoquinoline and quinazoline series. Ring substituents such as fluoro, bromo, nitro, acetyl, and aminomethyl on the isoquinoline ring resulted in a significant loss of activity. Likewise, similar groups on the quinazoline ring also reduced inhibitory activity. However, the 6- and 7-aminoquinazoline derivatives, 75 and 76, were potent inhibitors, with IC(50) values in the TNFalpha in vitro assay of approximately 5 microM for each. An in vivo mouse model of pulmonary inflammation was then used to evaluate promising candidate compounds identified in the primary in vitro assay. Compound 75 was selected for further study in this inhalation model, and was found to reduce the level of TNFalpha in brochoalveolar lavage fluid of LPS-treated mice by about 50% that of control mice. Thus, compounds such as 75, which can effectively inhibit proinflammatory

cytokines such as TNFalpha in clinically relevant animal models of inflammation and fibrosis, may have potential as new antiinflammatory agents. Finally, a quinazoline derivative suitable to serve as a photoaffinity radiolabeled compound was prepared to help identify the putative cellular target(s) for these TNFalpha inhibitors.

Clary JJ, Feron VJ, Van Velthuijsen JA. **Safety assessment of lactate esters.** Regul Toxicol Pharmacol 1998 Apr;27(2):88-97.
Lactate esters have an oral LD50 greater than 2000 mg/kg and the inhalation LC50 is generally above 5000 mg/m3 and they may be potential eye and skin irritants, but not skin sensitizers. No evidence of teratogenicity or maternal toxicity was observed in an inhalation (2-ethylhexyl-l-lactate) or dermal study (ethyl-l-lactate). Subacute inhalation studies have been conducted at concentration up to 600 mg/m3 or higher on four lactate esters (ethyl, n-butyl, isobutyl, and 2-ethylhexyl-l-lactate). Degenerative and regenerative changes in the nasal cavity were noted in all studies. The NOAEL in ethyl, n-butyl, and isobutyl-l-lactate vapor studies was 200 mg/m3. For aerosol exposure, 2-ethylhexyl-l-lactate, the most toxic of the lactates, minimal damage to the nasal epithelium was noted at 75 mg/m3 with vapor being slightly less toxic than the aerosol. Lactates do not appear to cause systemic toxicity, except at very high concentrations (1800 mg/m3 or higher). These systemic effects may be secondary to severe irritation seen at high doses. Sensory irritation tests suggest that a vapor exposure limit of 75 mg/m3 (approximately 15 ppm) should prevent irritation in humans and therefore an occupational exposure level for vapor of 75 mg/m3 is recommended. However, aerosol exposure should be kept as low as possible. The low vapor pressure of the higher molecular weight esters would tend to keep vapor exposure low and the odor of lactate esters serves as a warning of exposure. These lactate esters are readily biodegradable, suggesting little concern from an environmental point of view. Copyright 1998 Academic Press.

Claycamp HG, Sussman NB. **A simple inter-class distance parameter for predictive SAR-QSAR models.** Quant Struct Activity Relat 1999;18(1):11-5.
BIOSIS COPYRIGHT: BIOL ABS. The objective of many SAR or QSAR experiments is to develop statistical classification models that can separate chemicals into classes of "active" or "inactive" under a toxicological endpoint. Several existing statistical methods are often used to provide either quantitative or qualitative measures of the distance or separation between the active and inactive classes, including sensitivity, accuracy and the receiver-operator characteristic (ROC) curve. The present study proposes a simple "distan.

Dai J, Sun C, Han S, Wang L. **QSAR for polychlorinated organic compounds (PCOCs). I. Prediction of partition properties for PCOCs using quantum chemical parameters.** Bull Environ Contam Toxicol 1999;62(5):530-8.

De Groot MJ, Ackland MJ, Horne VA, Alex AA, Jones BC. **Novel approach to predicting P450-mediated drug metabolism: development of a combined protein and pharmacophore model for CYP2D6.** J Med Chem 1999;42(9):1515-24.
A combined protein and pharmacophore model for cytochrome P450 2D6 (CYP2D6) has been derived using various computational chemistry techniques. A combination of pharmacophore modeling (using 40 substrates), protein modeling, and molecular orbital calculations was necessary to derive a model

which incorporated steric, electronic, and chemical stability properties. The initial pharmacophore and protein models used to construct the combined model were derived independently and showed a high level of complementarity. The combined model is in agreement with experimental results concerning the substrates used to derive the model, with site-directed mutagenesis data available for the CYP2D6 protein, and takes into account the site-directed mutagenesis results for a variety of other 2-family P450s.

De Julian-Ortiz JV, Galvez J, Munoz-Collado C, Garcia-Domenech R, Gimeno-Cardona C. **Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds.** J Med Chem 1999;42(17):3308-14.
The activity of new anti-HSV-1 chemical structures, designed by virtual combinatorial chemical synthesis and selected by a computational screening, is determined by an in vitro assay. A virtual library of phenol esters and anilides was formed from two databases of building blocks: one with carbonyl fragments and the other containing both substituted phenoxy and phenylamino fragments. The library of virtually assembled compounds was computationally screened, and those compounds which were selected by our mathematical model as active ones were finally synthesized and tested. Our antiviral activity model is a "tandem" of four linear functions of topological graph-theoretical descriptors. A given chemical structure was selected as active if it satisfies every discriminant equation in that model. The final result was that five new structures were selected, synthesized, and tested: all of them demonstrated activity, and three showed appreciable anti-HSV-1 activity, with IC(50) values of 0.9 &mgr;M. The same model, applied to a database of known compounds, has identified the anti-herpes activity of the following compounds: 3,5-dimethyl-4-nitroisoxazole, nitrofurantoin, 1-(pyrrolidinocarbonylmethyl)piperazine, nebularine, cordycepin, adipic acid, thymidine, alpha-thymidine, inosine, 2, 4-diamino-6-(hydroxymethyl)pteridine, 7-(carboxymethoxy)-4-methylcoumarin, 5-methylcytidine, and others that showed less activity.

Dove S, Buschauer A. **Imidazolylpropylguanidines as histamine H2 receptor agonists: 3D-QSAR of a large series**. Pharm Acta Helv 1998;73(3):145-55.
IPA COPYRIGHT: ASHP The three-dimensional (3D) quantitative structure activity relationship (QSAR) of a large series of 141 imidazolylpropylguanidines with histamine H2 receptor agonist activity, which were submitted to comparative molecular field analysis (CoMFA), is described.

Eldred DV, Jurs PC. **Prediction of acute mammalian toxicity of organophosphorus pesticide compounds from molecular structure.** SAR QSAR Environ Res 1999;10(2-3):75-99.
A quantitative structure-activity relationship (QSAR) investigation was done for the acute oral mammalian toxicity (LD50) of a set of 54 organophosphorus pesticide compounds. The compounds were represented with calculated molecular structure descriptors, which encoded their topological, electronic, and geometrical features. Feature selection was done with a genetic algorithm to find subsets of descriptors that would support a high quality computational neural network (CNN) model to link the structural descriptors to the -log(mmol/kg) values for the compounds. The best seven-descriptor non-linear CNN model found had an rms error of 0.22 log units for the training set compounds and 0.25 log units for the prediction set compounds.

Eldred DV, Weikel CL, Jurs PC, Kaiser KL. **Prediction of fathead minnow acute toxicity of organic compounds from molecular structure.** Chem Res Toxicol 1999;12(7):670-8.

Interest in the prediction of toxicity without the use of experimental data is growing, and quantitative structure-activity relationship (QSAR) methods are valuable for such predictions. A QSAR study of acute aqueous toxicity of 375 diverse organic compounds has been developed using only calculated structural features as independent variables. Toxicity is expressed as -log(LD(50)) with the units -log (millimoles per liter) and ranges from -3 to 6. Multiple linear regression and computational neural networks (CNNs) are utilized for model building. The best model is a nonlinear CNN model based on eight calculated molecular structure descriptors. The root-mean-square log(LD(50)) errors for the training, cross-validation, and prediction sets of this CNN model are 0.71, 0.77, and 0.74 -log(mmol/L), respectively. These results are compared to a previous study with the same data set which included many more descriptors and used experimental data in the descriptor pool.

Farag HH, Omar FA, Mahfouz NM. **Molecular structural parameters in tuberculostatic activity of polyhydroxyxanthones**. Bull Pharm Sci Assiut Univ 1998;21(1):7-14.
IPA COPYRIGHT: ASHP The tuberculostatic activity of a series of 7(2)-polyhydroxyxanthones was studied in vitro as a function of lipophilic parameters and some molecular orbital descriptors. Quantitative structure-activity relationship analysis demonstrated an increase in the lipophilic character by itself that did not correlate with increased potency against Mycobacterium lufu. A permeability study using a standard cellophane membrane showed that the synthesized compounds were poorly penetrating and may act directly on the cell wall. On the contrary, higher correlation was observed between the activity parameter (log ka*) and the electron density at C-4.

Felton JS, Knize MG, Hatch FT, Tanga MJ, Colvin ME. **Heterocyclic amine information and the impact of structure on their mutagenicity.** Cancer Lett 1999;143(2):127-34.
The occurrence and formation of heterocyclic amines in foods is discussed in light of the consistent finding of a new class of imidazopyridines. In addition, a quantitative structure-activity relationship will be presented correlating the potency of these imidazopyridines to predicted chemical properties. Although no strong linear correlation is found between the potency and the chemical properties, a low dipole moment is found to be a qualitative predictor of high mutagenic potency.

Greene N, Judson PN, Langowski JJ, Marchant CA. **Knowledge-based expert systems for toxicity and metabolism prediction: DEREK, StAR and METEOR.** SAR QSAR Environ Res 1999;10(2-3):299-314.
It has long been recognised that the ability to predict the metabolic fate of a chemical substance and the potential toxicity of either the parent compound or its metabolites are important in novel drug design. The popularity of using computer models as an aid in this area has grown considerably in recent years. LHASA Limited has been developing knowledge-based expert systems for toxicity and metabolism prediction in collaboration with industry and regulatory authorities. These systems, DEREK, StAR and METEOR, use rules to describe the relationship between chemical structure and either toxicity in the case of DEREK and StAR, or metabolic fate in the case of METEOR. The rule refinement process for DEREK often involves assessing the predictions for a novel set of compounds and comparing them to their biological assay results as a measure of the system's performance. For example, 266 non-congeneric chemicals from the National Toxicology Program database have been processed through the DEREK mutagenicity knowledge base and the predictions compared to their Salmonella typhimurium mutagenicity data. Initially, 81 of 114 mutagens (71%) and 117 of 152 non-mutagens (77%) were

correctly identified. Following further knowledge base development, the number of correctly identified mutagens has increased to 96 (84%). Further work on improving the predictive capabilities of DEREK, StAR and METEOR is in progress.

Gute BD, Grunwald GD, Basak SC. **Prediction of the dermal penetration of polycyclic aromatic hydrocarbons (PAHs): a hierarchical QSAR approach.** SAR QSAR Environ Res 1999;10(1):1-15.
Attempts were made to develop hierarchical quantitative structure-activity relationship (QSAR) models for the dermal penetration of polycyclic aromatic hydrocarbons (PAHs) using four classes of theoretical structural parameters; viz., topostructural, topochemical, geometric, and quantum chemical descriptors; and physicochemical properties such as molecular weight (MW) and lipophilicity (log P--octanol/water). The results show that topostructural, topochemical, and geometric descriptors and molecular weight are equally effective in predicting the dermal penetration of PAHs. Quantum chemical parameters did not make any improvements in the predictive power of the QSAR models.

Hirashima A, Shinkai K, Pan C, Kuwano E, Taniguchi E, Eto M. **Quantitative structure-activity studies of octopaminergic ligands against Locusta migratoria and Periplaneta americana.** Pestic Sci 1999;55(2):119-28.
BIOSIS COPYRIGHT: BIOL ABS. The quantitative structure-activity relationship (QSAR) of octopaminergic agonists and antagonists against the thoracic nerve cord of the migratory locust, Locusta migratoria, was analysed using physicochemical parameters and regression analysis. Sixty-five molecules that employ a single (3H)octopamine (OA) binding criterion were selected. The hydrophobic and electronic nature of the ligands were the most important factors for binding activity: the positive log of octanol-water partition coeffic ATs), arylethanolamines (AEAs) and 1-(substituted-phenyl) imidazolidine-2-thiones (SPITs) in stimulating adenylate cyclase prepared from thoracic nerve cords of the American cockroach Periplaneta americana was examined using parameters calculated by molecular orbital. The hydrophobicity, spatial descriptor and conformational similarity index were the most important factors for adenylate-cyclase activation: the positive solvent-accessible surface (SAS) area, log P and negative conformational energ.

Ince NH, Dirilgen N, Apikyan IG, Tezcanli G, Ustun B. **Assessment of toxic interactions of heavy metals in binary mixtures: a statistical approach.** Arch Environ Contam Toxicol 1999;36(4):365-72.
BIOSIS COPYRIGHT: BIOL ABS. Toxicity of zinc, copper, cobalt, and chromium ions and their binary interactions were studied at varying test levels by using a battery of two tests, Microtox and duckweed with Vibrio fisheri and Lemna minor as test organisms, respectively. The type of toxic interaction at each test combination was assessed by a statistical approach based on testing the null hypothesis of "additive toxicity" at 95% confidence level. The interactions were called "antagonistic," "additive," or "synergistic" in ac h higher in Microtox responses than in those of duckweed. Finally, synergism was found to be a rare interaction in Microtox results, but totally unlikely in duckweed within the selected test combinations.

Ishikawa Y. **A recessive lethal mutation, tb, that bends the midbrain region of the neural tube in the early embryo of the medaka.** Neurosci Res 1996 Feb;24(3):313-7.
A recessive lethal mutation, tb (twisted brain), in the homozygous embryo of which the neural tube is twisted, was newly found in the medaka (Oryzias latipes). The mutation was recovered in the progeny of

a male medaka which had been treated with N-ethyl-N-nitrosourea (ENU). The mutation affected morphogenic cell movements (extension and convergence) in the gastrula and neurula stages. In these stages, the embryonic body was shorter and the convergence of the neuroectodermal cells proceeded more slowly, especially in the prospective midbrain region, in the mutant embryo than in the wild-type embryo. As the neural rod formed in the mutant embryo, it curved in the midbrain region, usually protruding to the right as viewed from above. Structures lying anterior and posterior to the midbrain region were invariably present and developed fairly normally in the mutant embryo. These results show that there exists a genetically separable component in the developmental process of the formation of a straight and symmetrical neural tube.

Ishikawa Y, Hyodo-Taguchi Y. **Heritable malformations in the progeny of the male medaka (Oryzias latipes) irradiated with X-rays.** Mutat Res 1997 Mar 17;389(2-3):149-55.
Heritable malformations were examined in the progeny of X-irradiated male medaka (Oryzias latipes) by three-generation crosses. Two X-irradiated male fish were pair-mated with non-irradiated females to produce F1 founders, and each F1 fish was pair-mated with a non-irradiated fish to produce F2 progeny. For detection of recessive mutations, pair-matings between F2 siblings were performed for each F1 family. Morphogenesis of the embryos of each generation was observed using a stereomicroscope, throughout the entire period of embryonic development. In the F1 embryos, the frequencies of dominant lethals and malformations were increased by the X-irradiation. Two out of 30 F1 pairs produced a number of malformed and lethal F2 embryos, indicating inheritance of high rates of the dominant lethals in the two F1 families. Moreover, F2 sib-pairs offspring of which exibited high rates of dominant lethals were found in 10 out of 28 F1 families. Recessive lethal mutations, which were associated with a particular phenotype, were found in 2 out of the 28 F1 families. These results indicate that the heritable malformations induced by X-irradiation can be studied in the medaka.

Lewis DF, Dickins M, Lake BG, Eddershaw PJ, Tarbit MH, Goldfarb PS.**Molecular modelling of the human cytochrome P450 isoform CYP2A6 and investigations of CYP2A substrate selectivity.** Toxicology 1999;133(1):1-33.
(1) The generation of a homology model of CYP2A6, the major catalyst of human hepatic coumarin 7-hydroxylase activity, involves the use of the recently published substrate-bound CYP102 crystal structure as a template. (2) A substantial number of structurally diverse CYP2A6 substrates are found to dock satisfactorily within the putative active site of the enzyme, leading to the formulation of a structural template (or pharmacophore) for CYP2A6 specificity/selectivity. (3) The CYP2A6 model is consistent with available evidence from site-directed mutagenesis studies carried out on CYP2A subfamily isoforms, and enables some explanation of species differences in CYP2A-mediated metabolism of certain substrates. (4) Quantitative structure-activity relationship (QSAR) analysis of CYP2A5 (the mouse orthologue) mutants yields statistically significant correlations between various properties of amino acid residues and coumarin 7-hydroxylase activity.

Lipnick RL. **Correlative and mechanistic QSAR models in toxicology.** SAR QSAR Environ Res 1999;10(2-3):239-48.
An outline is provided on the development and use of correlative and mechanistic approaches to predictive toxicology, with particular emphasis on the experience at the U.S. EPA as applied to assessing the potential hazard posed by new industrial chemicals for which little or no test data are

provided under the Toxic Substances Control Act. This information is presented with a historical perspective.

Motohashi N, Yamagami C, Tokuda H, Konoshima T, Okuda Y, Okuda M, Mukainaka T, Nishino H, Saito Y. **Inhibitory effects of dehydrozingerone and related compounds on 12-O-tetradecanoylphorbol-13-acetate induced Epstein-Barr virus early antigen activation.** Cancer Lett 1998;134(1):37-42.
Dehydrozingerone, 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, is half an analog of curcumin which is known to have anti-tumor activity. The anti-tumor promoting activity of dehydrozingerone was evaluated by determining the inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). The concentration needed for 50% inhibition of the tumor promotion (IC50) of dehydrozingerone was similar to that of curcumin. To elucidate the structure-activity relationship on the anti-tumor promoting activity, dehydrozingerone, curcumin, isoeugenol, which has no carbonyl group in the side chain, benzalacetone, which is the basic structure of dehydrozingerone, o-dehydrozingerone, which is the ortho-hydroxyl substituted compound of dehydrozingerone, and their related compounds were investigated using the in vitro short-term assay on TPA-induced EBV-EA activation. o-Dehydrozingerone showed the most potent inhibitory effect in a series of tested dehydrozingerone derivatives and their related monosubstituted benzalacetones. This suggests that the occupation at both ortho positions of the hydroxyl group enhances the anti-tumor promoting activity. Isoeugenol inhibited the tumor promoting activity at a concentration of about one-third of the IC50 of dehydrozingerone. This indicates that the carbonyl group in the side chain has a negative impact on the anti-tumor promoting activity. The inhibitory effects of the carbon-carbon bond in the side chain were studied using benzylacetone with a single bond, benzalacetone with a double bond and 4-phenyl-3-butyn-2-one with a triple bond. 4-Phenyl-3-butyn-2-one inhibited the most potent activity followed by benzalacetone and benzylacetone.

Muresan A, Palage M, Arama O, Simiti I. **[Quantitative relations between chemical structure and antimicrobial activity (QSAR) in quaternary ammonium compounds and thiazolic nitrones series]**. Farmacia 1998 Mar-Apr;46:35-41. (Rum)

Nakagawa Y, Smagghe G, Kugimiya S, Hattori K, Ueno T, Tirry L, Fujita T. **Quantitative structure-activity studies of insect growth regulators: XVI. Substituent effects of dibenzoylhydrazines on the insecticidal activity to Colorado potato beetle Leptinotarsa decemlineata.** Pestic Sci 1999;55(9):909-18.
BIOSIS COPYRIGHT: BIOL ABS. Insecticidal activity against the Colorado potato beetle, Leptinotarsa decemlineata, was measured for a series of substituted N-tert-butyl-dibenzoylhydrazines, in which one of the benzoyl moieties closer to the tert-butyl group was fixed as being 2-chloro-substitued and the other variously substituted singly or doubly. The effects of substituents on the activity were quantitatively analysed using the classical quantitative structure-activity relationship (QSAR) procedure. The activity against th hasic contribution of the molecular hydrophobicity to activity against coleopterous larvae is themost conspicuous difference in substituent effects from those found for similar compounds against lepidopterous pest insects, and may be the basis of the variations in the activity spectrum for certain compounds in this series. The introduction ofbulkier substituents into the meta- and para-positions of the benzene ring, apart from the tert-butyl group, is unfavorable to activity. LD50

values against.

Parakulam RR, Lesniewski ML, Taylor-McCabe KJ, Tsai C. **QSAR studies of antiviral agents using molecular similarity analysis and structure-activity maps.** SAR QSAR Environ Res 1999;10(2-3):175-206.
Quantitative structure-activity relationships (QSAR) were developed for nucleoside analogs with anti-HIV activity. These compounds were investigated to determine the correlation of structure and toxicity/activity using molecular similarity analysis and structure-activity maps. A multiple-formula approach was used to perform quantitative molecular similarity analysis (QMSA) and QSAR study. Molecular descriptors such as number of atoms and bonds of a molecule (NAB), maximum common substructure (MaCS), and molecular similarity index (MSI) were used in our structure-activity relationship study. The MaCS of two molecules is defined as the substructure with the greatest NAB value common to both molecules. The MSI of two molecules X and Y is defined as MSI(X,Y) = [MaCS(X,Y)/NAB(X)] x [MaCS(X,Y)/NAB(Y)]. MaCS and MSI quantify the similarity between two molecular structures. Structure-activity maps (structure-toxicity map and structure-antiviral map) and QMSA were used to determine the site and type of modification for reduced toxicity and improved activity of new compounds.

Piskorska-Pliszczynska J. **[SAR and QSAR methods in the study of dioxin action]**. Rocz Panstw Zakl Hig 1998;49(4):433-45. (Pol)
Thousand chemicals are present within our environment, and for many of them, there is a little reliable information detailing their relative hazard. Added to that increasing concern over the use of animals in toxicity testing, high costs of these tests has made the search for and validation of alternative methods to predict the hazard and the relative risk of xenobiotics. QSAR (quantitative structure activity relationship) attempt to relate statistically the biological activity of compound with its physicochemical and structural properties. QSAR methods are often seen as the first step for valid toxicological prediction. Halogenated aromatic hydrocarbons typified by polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF) and biphenyls (PCBs) have been identified as residues in almost every component of the ecosystem. Some chemicals in this class cause adverse biological effects after binding to an intracellular cytosolic protein called the Ah receptor (AhR). Because of importance of the Ah receptor in determining toxicity, there have been a number of attempts to model the relationship between receptor binding and structure of xenobiotics. QSAR have found wide use in correlating the bioactivity of dioxins and related compounds with many kinds of biological entities. This paper will briefly summarise some SAR and QSAR study for halogenated aromatics as ligand for Ah receptor and the characteristic biological and toxic responses elicit by this class of chemicals. These study strongly support the role of AhR in dioxin toxicity.

Ramos E, Vaes W, Mayer P, Hermens J. **Algal growth inhibition of Chlorella pyrenoidosa by polar narcotic pollutants: toxic cell concentrations and QSAR modeling.** Aquatic Toxicol 1999;46(1):1-10.
BIOSIS COPYRIGHT: BIOL ABS. The effects of 11 polar narcotic pollutants (phenols, nitrobenzenes and anilines) on the algae Chlorella pyrenoidosa have been investigated in 72 h population growth inhibition tests. The lowest observed effect concentration and no-observed effect concentrations were determined. The partial effect concentrations were estimated (EC10 and EC50) by the Weibull function,

and no-effect concentrations were determined by using the DEBTOX program. In addition, for four of the chemicals, the internal cel entrations have been used to construct quantitative structure-activity relationship models using hydrophobicity and hydrogen bonding capacity descriptors. The models indicate that toxicity increases with hydrophobicity, good hydrogen bonding donor capacity, and low hydrogen bonding acceptor capacity. The models can be interpreted based on the composition of biomembranes, which are supposed to be the target of narcotic pollutants.

Rosenkranz HS, Cunningham AR, Zhang YP, Claycamp HG, Macina OT, Sussman NB, Grant SG, Klopman G. **Development, characterization and application of predictive-toxicology models.** SAR QSAR Environ Res 1999;10(2-3):277-98.
The adoption of SAR techniques for risk assessment purposes requires that the predictive performance of models be characterized and optimized. The development of such methods with respect to CASE/ MULTICASE are described. Moreover, the effects of size, informational content, ratio of actives/ inactives in the model on predictivity must be determined. Characterized models can provide mechanistic insights: nature of toxicophore, reactivity, receptor binding. Comparison of toxicophores among SAR models allows a determination of mechanistic overlaps (e.g., mutagenicity, toxicity, inhibition of gap junctional intercellular communication vs. carcinogenicity). Methods have been developed to combine SAR submodels and thereby improve predictive performance. Now that predictive toxicology methods are gaining acceptance, the development of Good Laboratory Practices is a further priority, as is the development of graduate programs in Computational Toxicology to adequately train the needed professional.

Rosenkranz HS, Cunningham AR, Zhang YP, Klopman G. **Applications of the case/multicase SAR method to environmental and public health situations.** SAR QSAR Environ Res 1999;10(2-3):263-76.
The availability of validated and characterized SAR models of toxicological phenomena provides a method to apply SAR technology to a variety of environmental, public health and industrial situations. These include (i) the prioritization of environmental pollutants for control and/or regulation, (ii) the design of multi-action optimized therapeutics from which the potential for unwanted side-effects have been engineered out, (iii) the development of SAR-based computer-driven screening procedure to identify candidate therapeutics based upon combinatorial chemistry or compilations of molecular structures, (iv) the generation of toxicological profiles to be used in the selection of benign chemicals in the early stages of product development.

Rybczynski PJ, Combs DW, Jacobs K, Shank RP, Dubinsky B.**gamma-Aminobutyrate-A receptor modulation by 3-aryl-1-(arylsulfonyl)- 1,4,5,6-tetrahydropyridazines.** J Med Chem 1999;42 (13):2403-8.
A series of 3-aryl-1-(arylsulfonyl)-1,4,5,6-tetrahydropyridazine allosteric modulators of the GABAA receptor was synthesized, and biological activity was examined in vitro and in vivo. Beginning with 1a, stepwise modification of the substituents and conservation of the scaffold yielded a chemical series in which the modulatory activity was enhanced by the presence of GABA. The SAR suggests, but does not establish, that the compounds bind to the steroid binding site on the GABAA receptor. The GABA shift for each compound indicates that all compounds in this series are either agonists or partial agonists.

Schulten HR. **Interactions of dissolved organic matter with xenobiotic compounds: molecular modeling in water.** Environ Toxicol Chem 1999;18(8):1643-55.
BIOSIS COPYRIGHT: BIOL ABS. An hypothesis for the structure of dissolved organic matter (DOM) in water is proposed. It is based on previously published humic acid and soil organic matter (SOM) models. Personal computer (PC)-based molecular modeling and geometry optimization of DOM and humic/xenobiotic complexes in vacuo and water were performed using modern PC software in order to determine low energy conformations and to simulate site-specific processes such as trapping and binding of biological and anthropogenic substanc were determined by quantitative structure-activity relationship software. Focal points were molecular properties, such as solvent accessibility as well as van der Waals surface areas and volumes, partial charges, hydration energy (peptides), hydrophobicity (log P), refractivity, and polarizabilities of humic/xenobiotic complexes were determined. Molecular mechanics calculations show that nonbonded forces (e.g., van der Waals) and hydrogen bonds were the main reasons for temporary immobility of.

Takanashi Y, Higashiyama K, Komiya H, Takayama K, Nagai T. **Thiomenthol derivatives as novel percutaneous absorption enhancers**. Drug Dev Ind Pharm 1999;25(1):89-94.
IPA COPYRIGHT: ASHP Thirteen thiomenthol derivatives were synthesized and their ability to promote the percutaneous absorption of ketoprofen from hydrogels was studied in rats. As an index of promoting activity of the derivatives, an enhancement factor (Ef) was defined as the ratio of the apparent penetration rate with enhancer to the value obtained without enhancer. Total irritation score was estimated by summation of each irritation score in several parts of the skin. Physicochemical parameters of thiomenthol derivatives were calculated and the quantitative relationships between these parameters and Ef or total irritation score were determined on the basis of multiple regression analysis. As a result, a parabolic relationship between partition coefficient (log P) and Ef was noted. A similar relationship was observed for total irritation score.

Thomsen M, Rasmussen AG, Carlsen L. **SAR/QSAR approaches to solubility, partitioning and sorption of phthalates.** Chemosphere 1999;38(11):2613-24.
BIOSIS COPYRIGHT: BIOL ABS. The environmental behavior of xenobiotics such as phthalates is closely connected to parameters such as are solubility (Cwsat), partitioning (e.g. Kow) and sorption (e.g. Koc). In this paper the solubility, octanol-water partitioning and sorption of a series of phthalic acid esters are described through various SAR/QSAR concepts including molecular connectivity indices (MCI), electrotopological atomic state indices (EASI) and group-contribution (UNIFAC). Advantages and disadvantages of MCI and U xtent solubility are, according to EASI analyses, well correlated to hydrophobicity as expressed by the "alkyl- group" electrotopological index. The latter correlations is further improved by introducing the polar component as expressed by the "ester- group" electrotopological index.

Tmej C, Chiba P, Huber M, Richter E, Ecker G, et al. **Combined Hansch/Free-Wilson approach as predictive tool in QSAR studies on propafenone-type modulators of multidrug resistance**. Arch Pharm 1998 Jul-Aug;331:233-40.

Tuppurainen K. **Frontier orbital energies, hydrophobicity and steric factors as physical QSAR**

descriptors of molecular mutagenicity. A review with a case study: MX compounds. Chemosphere 1999;38(13):3015-30.
A review on QSARs (Quantitative Structure-Activity Relationships) in modelling molecular mutagenicity is given. The importance of hydrophobicity, frontier orbital (HOMO and LUMO) energies and steric factors as physical descriptors of mutagenicity is emphasized. In addition, some possible connections between QSAR models and the general electrophilic theory of genotoxic activity are discussed. As a detailed example, QSARs for the Ames Salmonella typhimurium TA100 mutagenicity of halogenated hydroxyfuranones including MX, one of the most potent bacterial mutagens ever identified, are discussed and a plausible mechanism for their mutagenic activity is proposed.

Vedani A, Zbinden P. **Quasi-atomistic receptor modeling: bridge between 3D QSAR and receptor fitting**. Pharm Acta Helv 1998;73(1):11-8.
IPA COPYRIGHT: ASHP To derive semi-quantitative structure-activity relationships for a series of 6 receptor systems, quasi-atomistic receptor modeling was used. Quasi-atomistic receptor modeling was able to predict the relative free energies of ligand binding of an independent set of test ligands within 0.55 to 0.94 kcal/mol of their experimental value, corresponding to an uncertainty in the binding affinity of a factor of 2.5 to 5.

Wang S, Liu M, Lewin NE, Lorenzo PS, Bhattacharrya D, Qiao L, Kozikowski AP, Blumberg PM. **Probing the binding of indolactam-V to protein kinase C through site-directed mutagenesis and computational docking simulations.** J Med Chem 1999;42(18):3436-46.
Protein kinase C (PKC) comprises a family of ubiquitous enzymes transducing signals by the lipophilic second messenger sn-1, 2-diacylglycerol (DAG). Teleocidin and its structurally simpler congener indolactam-V (ILV) bind to PKC with high affinity. In this paper, we report our computational docking studies on ILV binding to PKC using an automatic docking computer program, MCDOCK. In addition, we used site-directed mutagenesis to assess the quantitative contribution of crucial residues around the binding site of PKC to the binding affinity of ILV to PKC. On the basis of the docking studies, ILV binds to PKC in its cis-twist conformation and forms a number of optimal hydrogen bond interactions. In addition, the hydrophobic groups in ILV form "specific" hydrophobic interactions with side chains of a number of conserved hydrophobic residues in PKC. The predicted binding mode for ILV is entirely consistent with known structure-activity relationships and with our mutational analysis. Our mutational analysis establishes the quantitative contributions of a number of conserved residues to the binding of PKC to ILV. Taken together, our computational docking simulations and analysis by site-directed mutagenesis provide a clear understanding of the interaction between ILV and PKC and the structural basis for design of novel, high-affinity, and isozyme-selective PKC ligands.

Ward CJ, Codd GA. **Comparative toxicity of four microcystins of different hydrophobicities to the protozoan, Tetrahymena pyriformis.** J Appl Microbiol 1999;86(5):874-82.
Microcystins (MC) are a group of over 60 cyclic heptapeptide hepatotoxins produced by cyanobacteria. The 1-octanol/water partition coefficients (log P) of MC-LR, -LY, -LW and -LF have been estimated by HPLC to be 2.16, 2.92, 3.46 and 3.56, respectively. Their in vivo toxicities to Tetrahymena pyriformis was also investigated. Twenty-four hour LC50 values followed the order MC-LR > -LY > -LW approximately -LF. The LC50 values of MC-LR and -LY were significantly reduced in the presence of 1% (v/v) dimethylsulphoxide, although no significant effect occurred with MC-LW or -LF.

Tetrahymena pyriformis respiration rates were inhibited by MC-LR in both a time- and dose-dependent manner. Increasing log P of the MC used caused a significantly greater inhibition of respiration. Population growth rate and maximum culture density were inhibited by all MC variants in proportion to log P. Positive correlations between all toxicological endpoints and log P occurred, with the most hydrophobic toxin, MC-LF, being 1.4 to 3.5 times more toxic than MC-LR. MC-LW had a similar toxicity to MC-LF, while MC-LY toxicity was intermediate between that of MC-LR and -LF. Implications of this positive relationship between in vivo toxicity and hydrophobicity for the toxicity of MC to aquatic organisms, and the potential for using log P as a descriptor in a quantitative structure-activity relationship for MC, are discussed.

Warne MA, Osborn D, Lindon JC, Nicholson JK. **Quantitative structure-toxicity relationships for halogenated substituted-benzenes to Vibrio fischeri, using atom-based semi-empirical molecular-orbital descriptors.** Chemosphere 1999;38(14):3357-82.
Quantitative structure-toxicity relationships (QSTR's) are derived for an extensive series of halogenated benzenes, anilines, phenols, nitrobenzenes, toluenes and other substituted benzenes against Vibrio fischeri using a wide range of whole molecule and atom-based descriptors derived from semi-empirical molecular-orbital calculations. In terms of direct statistical correlation with toxicity it was found that the molar refractivity was the most important parameter, closely followed by the solvent accessible surface area of the compound. The accuracy of these descriptors in fitting the numerous fluoro- and chloro-mono-aromatic compounds was compared with bromine and iodine analogues, where the 'best' descriptors for the former were found in general to be less accurate for the latter in the case of multi-halogen substitution. The equations obtained were also used to classify the compounds into narcosis-based mechanisms of toxicity and those with respiratory uncoupling potential. A combination of the molar refractivity and the nucleophilic susceptibility of one of the meta ring carbons predicted the toxicity of the halo-benzenes and toluenes, along with anisoles, benzonitriles, nitrobenzenes and most of the anilines. The relevance of these descriptors to developing coherent and more generally applicable models for QSTR's of mono-aromatic compounds to other species in environmental toxicology is discussed.

Wu C, Johnson RK, Mattern MR, Wong JC, Kingston DG. **Synthesis of furanonaphthoquinones with hydroxyamino side chains.** J Nat Prod 1999;62(7):963-8.
Several furanonaphthoquinones have shown useful activity in a yeast assay for DNA-damaging agents and cytotoxicity in mammalian cell culture assays. These results, together with the planar aromatic character of the furanonaphthoquinones, suggested that they might be acting as DNA intercalators. In an attempt to improve this activity, various analogues containing a hydroxyamino side chain have been synthesized. The analogues were prepared by standard methods, but some unexpected reactions were observed nonetheless. Thus, 8-formyl-5-methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (24) showed an unusual reactivity toward reductive amination, with the reaction proceeding further to give one of two different cyclized products, depending on the amination reagent used. Bioassay results indicated that only simple furanonaphthoquines showed activity in a yeast assay for DNA-damaging agents; compounds with a substituted hydroxyamino side chain were uniformly inactive in this assay. Most of the compounds with a substituted hydroxyamino side chain on the furan ring did, however, show cytotoxicity, although none of them was any more active than the simple aldehyde 2-formyl-4, 9-

dihydronaphtho[2,3-b]furan-4,9-dione (14). This evidence tends to suggest that the furanonaphthoquinones do not serve primarily as DNA intercalators, because if this were the case, they would have been expected to show an increased activity on conversion to their hydroxyamino side chain derivatives.

Xia CQ, Yang JL, Ren S, Lien EJ. **QSAR analysis of polyamine transport inhibitors in L1210 cells**. J Drug Target 1998;6(1):65-77.
IPA COPYRIGHT: ASHP A quantitative structure-activity relationship (QSAR) approach was used in the construction of a mathematical model correlating the biological activities of 63 polyamine transport inhibitors in L1210 leukemia cells with their physicochemical parameters. The model provided some quantitative information about the relationship between the polyamines' function as transport inhibitors and their molecular structures.

Zidorn C, Stuppner H, Tiefenthaler M, Konwalinka G. **Cytotoxic activities of hypocretenolides from Leontodon hispidus.** J Nat Prod 1999;62(7):984-7.
The hypocretenolides are a small group of sesquiterpene lactones with an unusual ring structure and are constituents of several species from the tribe Lactuceae of the family Asteraceae. In the present communication we report on the cytotoxic effects of three hypocretenolides (1-3) from Leontodon hispidus on: (a) eight solid-tumor cell lines (A431, HEP2, MCF7, OVCAR3, SK28, SK37, SW872, ZR75-1), tested by a (3)H-thymidine incorporation assay; (b) two different leukemia cell lines (GTB, HL60), measured by a MTT assay; and (c) CD34(+) bone-marrow cells, assessed by scoring the number of colonies derived from primitive and late erythroid progenitors (BFU-E and CFU-E) as well as from granulocytic/macrophagic progenitor cells (CFU-GM). The aglycon 14-hydroxyhypocretenolide (1) exhibited pronounced activities, although its beta-D-glucoside (2) showed no activity, even at the highest concentration tested (2 microM). 14-Hydroxyhypocretenolide-beta-D-glucoside-4',14' '-hydroxyhypocretenoate (3), the ester of the glucoside esterified with the open-chain form of the aglycon, was the most potently cytotoxic substance and proved to be even more active than the positive-control substance helenalin.

Zlatkov A, Peikov P, Danchev N, Ivanov D, Tsvetkova B. **Synthesis, toxicological, pharmacological assessment, and in vitro bronchodilating activity of some 7-theophyllinylacetyloxyglycols**. Arch Pharm 1998 Oct;331:313-8.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Abbott BD, Birnbaum LS, Diliberto JJ. **Rapid distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to embryonic tissues in C57BL/6N mice and correlation with palatal uptake in vitro.** Toxicol Appl Pharmacol 1996 Nov;141(1):256-63.
2,3,7,8-Tetracholoridbenzo-p-dioxin (TCDD) is a developmentally toxic environmental contaminant capable of inducing cleft palate and hydronephrosis in embryonic C57BL/6N mice. In this study, the disposition of TCDD was determined in pregnant C57BL/6N mice in the 24 hr immediately following oral administration on Gestation Day (GD) 12. TCDD was detected in maternal blood, liver, and fat and in the placenta, embryonic liver, and palate within 30 min after dosing on GD 12. The levels peaked in

blood and placenta at 3 hr and in the other tissues at 8 hr. Levels of TCDD decreased slightly after 8 hr in embryonic liver and palate. In vitro systems were used to study the mechanisms of action of TCDD and in these models exposure is typically reported as concentration of TCDD in the culture medium. The present study is the first to allow a direct comparison of the level of TCDD in embryonic tissue after in vivo and in vitro exposures. Uptake of TCDD was determined in embryonic palatal organ culture and tissue levels were then expressed in comparable units for both in vivo and in vitro exposures. The data provide new information on distribution in the pregnant mouse and the embryo and also show that the palatal organ culture model provides a reasonable dosimetric representation of in utero exposure.

Andersson PL, Blom A, Johannisson A, Pesonen M, Tysklind M, Berg AH, Olsson PE, Norrgren L. **Assessment of PCBs and hydroxylated PCBs as potential xenoestrogens: in vitro studies based on MCF-7 cell proliferation and induction of vitellogenin in primary culture of rainbow trout hepatocytes.** Arch Environ Contam Toxicol 1999;37(2):145-50.
In the present study, four structurally diverse polychlorinated biphenyls (PCBs) were chosen from a set of 20 PCBs selected to represent the 154 tetra- through hepta-chlorinated biphenyls. The purpose was to determine estrogenic activities of the chosen PCBs and five of their hydroxylated derivatives (OH-PCBs). A human breast cancer cell line (MCF-7) and primary cultures of rainbow trout (Oncorhyncus mykiss) hepatocytes were used to determine estrogenic effects. The PCBs 2,2',4,6,6'-pentachlorobiphenyl (104) and 2,2',3, 4', 5,6,6'-heptachlorobiphenyl (188), and the hydroxylated PCBs 2,2', 4',6'-tetrachloro-4-biphenylol (4'-50), 2',4', 6'-trichloro-4-biphenylol (4'-30), 2',3,5, 5'-tetrachloro-4-biphenylol (4'-72), 2',3,3',5', 6'-pentachloro-4-biphenylol (4'-112), and 2',3,4',5, 6'-pentachloro-4-biphenylol (4'-121) significantly increased MCF-7 cell proliferation. The coaddition of hydroxytamoxifen, an estrogen antagonist, inhibited increased cell proliferation. The activity of the hydroxylated PCBs 4'-50 and 4'-30 was significantly higher at all nominal concentrations tested as compared to the corresponding PCB, viz., PCB 104. The hydroxylated PCBs 4'-50, 4'-30, 4'-72 and 4'-112 induced vitellogenin synthesis in rainbow trout hepatocytes. Significant differences were found in the MCF-7 system between the parent PCB and its hydroxylated derivative, viz., for 4'-50/4'-30 and 104, and in the rainbow trout hepatocyte assay between 4'-112 and 112, respectively. No activity was observed for PCB 58 in any of the two assays in the present study. Even though cells from two different species (human and fish) are used in the present study, the results obtained by the two methods agree fairly well. In both studies the hydroxylated PCBs were more active than the PCBs, and 4'-30 was the most active compound second only to 17beta-estradiol. http://link.springer-ny. com/link/service/journals/00244/bibs/37n2p145.html.

Arnold SF, Bergeron JM, Tran DQ, Collins BM, Vonier PM, Crews D, Toscano WA Jr, McLachlan JA. **Synergistic responses of steroidal estrogens in vitro (yeast) and in vivo (turtles).** Biochem Biophys Res Commun 1997 Jun 18;235(2):336-42.
Many environmental agents exert estrogenic activity. Previous studies from our laboratories demonstrated that certain combinations of environmental estrogens (i) reverse the sex of male turtle embryos in a synergistic manner (Bergeron et al., (1994) Environ. Hlth Perspect. 102, 780-782), and (ii) synergistically transactivate the human estrogen receptor (hER) in yeast and mammalian cells (Arnold et al., (1996) Science 272, 1489-1492). Because our findings with synthetic estrogens suggested that combinations of naturally-occurring steroidal estrogens might also produce synergistic activity of the

ER, we used the same model systems to measure the activity of combinations of steroidal estrogens. The activity of combinations of estrone, estradiol-17beta or estradiol-17alpha in yeast strains expressing hER was synergistic at submaximal concentrations of both estrogenic compounds. However, synergy was not observed with mixtures of estrogens when the concentration of one of the estrogens alone was maximally active in yeast. Ligand-binding assays in yeast performed with various radiolabeled estrogens suggested that multiple estrogens may interact with the receptor. The estrogen-dependent process of sex-reversal of turtle embryos incubated at a male-producing temperature was used to determine whether steroidal estrogens also had synergistic activity in vivo. In this instance, a combination of estriol and estradiol-17beta was effective in reversing the gonadal sex of turtle embryos from males to females in a synergistic manner. Our results suggest that the synergy of some combinations of estrogens, synthetic or steroidal, may play a role in the estrogen-dependent process of sexual development in certain species.

Bajoria R, Fisk NM. **Permeability of human placenta and fetal membranes to thyrotropin-stimulating hormone in vitro.** Pediatr Res 1998 May;43(5):621-8.
We determined the placental transfer of TSH in an in vitro model of dually perfused isolated lobule in 28 human term placentas by adding varying concentrations (5-60 microIU mL(-1)) of TSH as a single bolus dose to the closed maternal circulation. Transmembrane transfer of TSH was also studied by adding 45 microIU mL(-1) to the maternal or fetal compartment of a dual chamber of fetal membranes in culture. Passage of freely diffusible markers creatinine and antipyrine were also studied in this model. TSH concentration was measured by third generation chemiluminescence assay with a sensitivity of 10 mIU mL(-1). In the perfusion experiments, at physiologic concentrations the slow decline of TSH in the maternal circulation was associated with a small linear increase in fetal levels to 0.11 +/- 0.04% of initial dose at 2 h. The placental transfer rate was 0.08 microIU min(-1). Increasing maternal concentrations of TSH were associated with proportional increases in transfer rate (y = 0.002x; R2 = 0.99) and placental uptake (y = 0.01x; R2 = 0.97). The placental permeability of TSH was 2.4 x 10(-4) mL min(-1) g(-1) and was proportional to its coefficients of diffusion in water and molecular size. The transmembrane transfer and permeability of TSH was comparable to those of the placenta. We conclude that TSH crosses the human term placenta and fetal membranes sparingly.

Balaguer P, Francois F, Comunale F, Fenet H, Boussioux AM, Pons M, Nicolas JC, Casellas C. **Reporter cell lines to study the estrogenic effects of xenoestrogens.** Sci Total Environ 1999;233(1-3):47-56.
In order to characterize the estrogenic activity of chemicals, we established complementary in vitro recombinant receptor-reporter gene assays in stably transfected MCF-7 and HeLa cells. MCF-7 cells which express the endogenous estrogen receptor alpha (ER alpha) were stably transfected with only an estrogen-regulated luciferase gene. These cells enable the detection of compounds which bind to ER alpha or interfere with the induction of ER alpha mediated gene expression. Furthermore, HeLa cells, which do not express endogenous ERs, were transfected with an ER alpha or an ER beta construct together with an estrogen-regulated luciferase gene, or a chimeric GAL4-ER alpha receptor and the corresponding luciferase reporter gene. Finally, we tested these four cellular models as tools to check the estrogenic activities of several potential xenoestrogens and to detect estrogenic activity in wastewater sewage treatment effluents. In all of the models, nonylphenol mixture (NPm), 4n-nonylphenol (4nNP), 2,4'-DDE, 4,4'-DDE and wastewater sewage treatment effluent were active, while PCB mixture (Aroclor

1254), PCB 77, atrazine and lindane (gamma hexachlorocyclohexane) were inactive. Dioxin partially activates the estrogen receptor in MCF-7 cells while in HeLa-derived cell lines, it decreased the estrogenic-induced expression of luciferase.

Bass EL, Sistrun SN. **Effect of UVA radiation on development and hatching success in Oryzias latipes, the Japanese Medaka.** Bull Environ Contam Toxicol 1997 Oct;59(4):537-42.

Becchetti A, Whitaker M. **Lithium blocks cell cycle transitions in the first cell cycles of sea urchin embryos, an effect rescued by myo-inositol.** Development 1997 Mar;124(6):1099-107.
Lithium is a classical inhibitor of the phosphoinositide pathway and is teratogenic. We report the effects of lithium on the first cell cycles of sea urchin (Lytechinus pictus) embryos. Embryos cultured in 400 mM lithium chloride sea water showed marked delay to the cell cycle and a tendency to arrest prior to nuclear envelope breakdown, at metaphase and at cytokinesis. After removal of lithium, the block was reversed and embryos developed to form normal late blastulae. The lithium-induced block was also reversed by myo- but not epi-inositol, indicating that lithium was acting via the phosphoinositide pathway. Lithium microinjection before fertilization caused arrest prior to nuclear envelope breakdown at much lower concentrations (3-5 mM). Co-injection of myo-inositol prevented the block. Microinjection of 1-2 mM lithium led to block at the cleavage stage. This was also reversed by coinjection of myo-inositol. Embryos blocked by lithium microinjection proceeded rapidly into mitosis after photolysis of caged inositol 1,4,5-trisphosphate. These data demonstrate that a patent phosphoinositide signalling pathway is essential for the proper timing of cell cycle transitions and offer a possible explanation for lithium's teratogenic effects.

Benson WH, Nimrod AC. **Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens.** Aquatic Toxicol 1998 Dec;44(1-2):141-56.
Japanese medaka were exposed to environmentally-relevant concentrations of environmental estrogens: nonylphenol (NP, 0.5, 0.8 and 1.9 ug/L), methoxychlor (MXC, 0.2, 0.6 and 2.3 ug/L) and estradiol (E2, 0.01, 0.12 and 1.66 ug/L). 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 treatmnet 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.

Bentivegna CS, Piatkowski T. **Effects of tributyltin on medaka (Oryzias latipes) embryos at different stages of development.** Aquatic Toxicol 1998 Dec;44(1-2):117-28.
Potential mechanisms of action for the toxicity of tributyltin (TBT) were studied in the freshwater fish embryos of medaka (Oryzias latipes). Toxic concentrations of TBT have been found in estuaries and freshwater systems, presumably due to their use as biocides in boat, antifoulant paints and in industry for plastics production. Medaka embryos were exposed to a single concentration of TBT at developmental stages that corresponed to the formation of structures and/or organs which might be potential targets. Times of exposure included day 0, oviposition, day 3, completion of somite formation, and day 5, liver

formation. Endpoints for evaluating toxicity were acute embryo lethality (96 h), rate of embryo development, hatching sucess, gross abnormalities, as well as hatchling eye diameter and number of somites. The clear chorion of medaka embryos allowed staging and in ova observations. Results showed that the acute toxicity of TBT was stage related. The 96 h LC50 (LC50: lowest concentration to cause 50% lethality in the test population) for embryos exposed on day 0 was 159 nM, which was lower than that for days 3 and 5, 360 and 340 nM, respectively. Subchronic endpoints showed that toxicity was concentration related and that embryos exposed on day 0 were more sensitive than those exposed on days 3 and 5. Lowest observable effect levels (LOELs) for hatching success were 36 nM for day 0 and 143 nM for days 3 and 5. LOELs for the combined effects of hatching success and gross abnormalities were 36 nM for day 0 and 71 nM for days 3 and 5. Developmental rate was slowed by TBT in a concentration-related manner; however, embryos treated with 36 and 71 nM were able to recover and hatch at the same time as controls. Types of gross abnormalities were similar regardless of day of exposure and consisted of tails bent at the tip, curled, and/or shortened. These abnormalities corresponded with statistically significant reductions in numbers of somites in all three age groups exposed to 71 nM (p less than 0.05).Although day 0 embryos were the most sensitive, the similar abnormalities for all 3 days of exposure indicated that TBT's toxicity was not due to effects on an age-dependent target but one present throughout embryo development.

Bichet S, Wenger RH, Camenisch G, Rolfs A, Ehleben W, Porwol T, Acker H, Fandrey J, Bauer C, Gassmann M. **Oxygen tension modulates beta-globin switching in embryoid bodies.** FASEB J 1999 Feb;13(2):285-95.
Little is known about the factors influencing the hemoglobin switch in vertebrates during development. Inasmuch as the mammalian conceptus is exposed to changing oxygen tensions in utero, we examined the effect of different oxygen concentrations on beta-globin switching. We used an in vitro model of mouse embryogenesis based on the differentiation of blastocyst-derived embryonic stem cells to embryoid bodies (EBs). Cultivation of EBs at increasing oxygen concentrations (starting at 1% O2) did not influence the temporal expression pattern of embryonic (betaH1) globin compared to the normoxic controls (20% O2). In contrast, when compared to normoxically grown EBs, expression of fetal/adult (betamaj) globin in EBs cultured at varying oxygen concentrations was delayed by about 2 days and persisted throughout differentiation. Quantitation of hemoglobin in EBs using a 2,7-diaminofluorene-based colorimetric assay revealed the appearence of hemoglobin in two waves, an early and a late one. This observation was verified by spectrophotometric analysis of hemoglobin within single EBs. These two waves might reflect the switch of erythropoiesis from yolk sac to fetal liver. Reduced oxygenation is known to activate the hypoxia-inducible factor-1 (HIF-1), which in turn specifically induces expression of a variety of genes among them erythropoietin (EPO). Although EBs increased EPO expression upon hypoxic exposure, the altered beta-globin appearance was not related to EPO levels as determined in EBs overexpressing EPO. Since mRNA from both mouse HIF-1alpha isoforms was detected in all EBs tested at different differentiation stages, we propose that HIF-1 modulates beta-globin expression during development.

Boekelheide K, Hall SJ, Richburg JH. **In vitro evaluation of Sertoli cell toxicants which target microtubules and decrease seminiferous tubule fluid formation.** In Vitro Mol Toxicol 1998;11 (4):309-14.

BIOSIS COPYRIGHT: BIOL ABS. By targeting Sertoli cell microtubules, a group of testicular toxicants, including colchicine, 2,5-hexanedione, and carbendazim, can disrupt microtuble-dependent transport within the Sertoli cell, leading to decreased seminiferous tubule fluid formation and germ cell death. A critical step in this pathogenetic sequence, the formation of seminiferous tubule fluid, can be visualized by secretion-induced movement of a lumenal oil droplet in an in vitro assay. Using this assay, 2,5-hexanedione and colchicine have previously been shown to inhibit seminiferous tubule fluid formation (Richburg et al., 1994). In this study, the effects of carbendazim, the toxic metabolite of the fungicide benomyl, were determined following both in vivo and in vitro exposure. Seminiferous tubules isolated from adult Sprague-Dawley rats gavaged with 400 mg/kg carbendazim showed significantly decreased lumenal oil droplet movement after exposure. The inhibitory effect of in vivo exposure on seminiferous tubule fluid formation could be replicated by in vitro incubation of control seminiferous tubules with 31 muM carbendazim. These results provide additional support for the hypothesis linking altered Sertoli cell microtubule function to decreased seminiferous tubule fluid formation, and validate the lumenal oil droplet method as a useful in vitro assay of seminiferous tubule fluid formation.

Borel IM, Freire SM, Rivera E, Canellada A, Binaghi RA, Margni RA. **Modulation of the immune response by progesterone-induced lymphocyte factors.** Scand J Immunol 1999 Mar;49(3):244-50.
Rat spleen and peripheral blood lymphocytes express progesterone receptors whose concentration is increased greatly during the early phase of pregnancy. After stimulation of progesterone the expression of receptors was augmented 2-3 times. When cells were cultured in the presence of progesterone they released a soluble factor that inhibited cellular immunoreactions (MLR, CRC) and cellular proliferation as measured by thymidine incorporation by spleen-cell culture. This factor also inhibited the synthesis of anti-DNP antibodies by a mouse hybridoma and diminished the proportion of cells in phase S. However, the percentage of asymmetric molecules produced by the hybridoma remained unaltered. These results support the hypothesis that soluble factors released by rat lymphocytes modulate the immune response of the mother and participate in the mechanism that protects the fetus against antipaternal antibodies.

Bramley TA, Menzies GS, Mcphie CA. **Effects of alcohol on the human placental GnRH receptor system.** Mol Hum Reprod 1999;5(8):777-83.
Isolation of human term placental membranes in the presence or absence of protease inhibitors indicated that protease inhibitors significantly reduced the amounts of [(125)I]-labelled gonadotrophin-releasing hormone (GnRH) binding to membrane GnRH-receptors in vitro by approximately 20%. This decrease was largely due to the ethanol used to dissolve the serine protease inhibitor, phenylmethylsulphonylfluoride (PMSF). Ethanol alone decreased the specific binding of [(125)I]-labelled GnRH isoform (IC(50), 7.9 +/- 0.8 mg/ml; n = 6) or agonist tracers (IC(50), 10.0 +/- 1.4 mg/ml; n = 6) to human placental membranes in a dose-dependent manner. Other alcohols also interfered with [(125)I]-GnRH isoform or agonist binding: inhibition increased with increasing carbon chain length and was dependent on the isomeric position of the hydroxyl group. Fractionation of term placental cytosol by gel chromatography demonstrated the presence of a high molecular weight fraction (approximately 60-70 kDa) which inhibited [(125)I]-GnRH binding to human placental membranes. However, placental cytosol fractions did not cross-react significantly with a specific anti-GnRH antibody. Surprisingly, re-assay of cytosol fractions in the presence of a cocktail of protease inhibitors generated a factor (molecular weight approximately 40-50 kDa) which did cross-react strongly with the GnRH antibody.

The generation of this factor was due to the ethanol solvent rather than to the protease inhibitors per se, as treatment of pooled 'latent' cytosol fractions with ethanol alone generated GnRH-like immunoactivity (irGnRH) which competed in parallel with GnRH standard. The amount of irGnRH generated depended on the concentration of ethanol added to the 'latent' cytosol fractions. However, ethanol had no effect on the assay in the absence of cytosol fraction, or with inactive cytosol fractions. Thus, ethanol can perturb the human placental GnRH/GnRH-receptor system in vitro in two distinct ways: by inhibition of GnRH binding to receptor, and by dissociation of complexed endogenous GnRH-like factor(s) from a GnRH-binding protein. It is postulated that high alcohol consumption in vivo may interfere with placental GnRH secretion/action and affect placental secretion of factors important to the establishment and maintenance of pregnancy.

Brent RL. **Reproductive and teratologic effects of low-frequency electromagnetic fields: a review of in vivo and in vitro studies using animal models.** Teratology 1999;59(4):261-86.
BIOSIS COPYRIGHT: BIOL ABS. In order to evaluate the reproductive risks of low-frequency electromagnetic fields (EMF), it is important to include epidemiological and animal studies in the evaluation, as well as the appropriate basic science information in developmental biology and teratology. This review presents a critical review of in vivo animal studies and in vitro tests, as well as the biological plausibility of the allegations of reproductive risks. In vitro or in vivo studies in nonhuman species can be used to study tions will be manifested in living organisms at term. Other aspects of reproductive failure such as abortion, infertility, stillbirth, and prematurity, cannot be addressed by in vitro or culture experiments. In fact, they are very difficult to design and interpret in nonprimate in vivo models. The biological plausibility some of the basic mechanisms involved in reproductive pathology were evaluated, concentrating primarily on the mechanisms involved in the production of birth defects. The studie udies dealing with the reproductive effects of EMF exposure are extensive. There are >70 EMF research projects that deal with some aspect of reproduction and growth. Unfortunately, a large proportion of the embryology studies used the chick embryo and evaluated the presence or absence of teratogenesis after 48-52 h of development. This is not a stage of development at which an investigator could determine whether teratogenesis occurred. The presence of clinically relevant teratogenesis can only d neurobehavioral development were predominantly negative and are therefore not supportive of the hypothesis that low-frequency EMF exposures result in reproductive toxicity.

Cameo M, Fontana V, Cameo P, Vauthay LG, Kaplan J, Tesone M. **Similar embryotoxic effects of sera from infertile patients and exogenous interferon-gamma on long-term in-vitro development of mouse embryos.** Hum Reprod 1999;14(4):959-63.
Circulating embryotoxic factors could be responsible for reproductive failures observed in patients suffering from recurrent spontaneous abortions (RSA) and endometriosis. The mouse bioassay has been widely used to detect such factors, since sera from these patients inhibit early embryonic development. This bioassay consists in the in-vitro culture of two-cell mouse embryos in the presence of different sera up to the blastocyst stage (72 h of culture). In the present study experiments were performed over long culture times (3-7 days), from two-cell to spreading stages, to determine the in-vitro effect of sera obtained from RSA or endometriosis patients, as well as the effect of interferon (INF)-gamma on embryo development. An embryotoxicity cut-off value of 45% blastocyst formation was established using control sera. When development to the blastocyst stage was considered only 25% of RSA and 20%

of endometriosis sera were embryotoxic. However, all RSA sera significantly inhibited hatching ($P < 0.05$) and spreading stages ($P < 0.01$). IFN-gamma (10 micrograms/ml) ($P < 0.001$) did not impair early embryo development, but significantly inhibited blastocyst spreading. These observations suggest that culture to advanced embryonic stages increases the sensitivity of the bioassay and that IFN-gamma alters in-vitro peri-implantation mouse embryo development.

Confer PD, Wolfe RE. **Developmental toxicity screens of military propellants using Hydra attenuata.** Toxicologist 1997 Mar;36(1 Pt 2):261-2.
In vitro developmental toxicity screens were performed to determine the developmental hazard indexes (A/D ratios) for liquid propellant XM46 (LP), ammonium dinitramide (ADN) and ammonium perchlorate (AP) using the hydra assay. Hydra attenuata is the most primitive invertebrate composed of complex tissues and organs and it is the highest form that has the capability for whole body regeneration. In the hydra assay, both adult Hydra attenuata and "artificial embryos", composed of disassociated Hydra attenuata cells, were exposed to each test compound to investigate potential developmental toxicity. The A/D ratios for LP, ADN and AP were determined to be 1.25, 2.14 and 1.71, respectively. By definition, a low A/D ratio (less than 3) predicts a test chemical being toxic to an embryo only at levels that will also cause toxic signs in the adult animal. Therefore, the A/D ratios for LP, ADN and AP demonstrate that these propellants should not be considered primary developmental toxins.

Courage-Maguire C, Bacon CL, Nau H, Regan CM. **Correlation of in vitro anti-proliferative potential with in vivo teratogenicity in a series of valproate analogues [published erratum appears in Int J Dev Neurosci 1997 Jul;15(4-5):693-4]**. Int J Dev Neurosci 1997 Feb;15(1):37-43.
The prediction that an anti-proliferative effect coupled with a pro-differentiative action will detect a neural tube teratogen has been validated by comparison of these in vitro endpoints with in vivo teratogenicity in a series of closely allied valproate structural analogues. The majority of the compounds significantly inhibited C6 glioma proliferation, the most potent compounds being ranked as octanoic acid > 2-propylhexanoic acid > or = 2-ethylhexanoic acid > or = valproic acid. The anti-proliferative potency of these compounds did not correlate strictly to their relative in vivo teratogenic potential. Valproic acid exhibited an anti-proliferative IC50 of 1.45 mM, whereas 2-propyl-2-pentenoic acid and 2-propyl-4-pentenoic acid were virtually indistinguishable, exhibiting significantly lower IC50 values of 2.5 and 2.55 mM, respectively. The concanavalin A lectin affinity assay was employed to establish whether an anti-proliferative action was coupled with an increased state of cell differentiation. In this lectin affinity assay, the most potent analogues to significantly attenuate the affinity of exposed C6 glioma cells for concanavalin A lectin-coated plastic included 2-butylhexanoic acid, 2-propyl-4-pentenoic acid, 2-propylhexanoic acid and 2-ethylhexanoic acid in a manner which can be related to their relative teratogenic potencies in vivo. All compounds screened positive in both the anti-proliferative and pro-differentiative assays exhibited in vivo exencephalic rates of 5-44%. These included valproic acid, 2-ethylhexanoic acid, 2-propylhexanoic acid and 2-butylhexanoic acid. It would appear that combined anti-proliferative and pro-differentiative screens provide a promising detection system for teratogenic status in a series of valproate analogues.

Daston GP. **The theoretical and empirical case for in vitro developmental toxicity screens, and potential applications.** Teratology 1996 Jun;53(6):339-44.

In vitro assays for the screening of developmental toxicity potential have been under development for approximately 15 years. During that period, we have learned that assays consisting of primary cultures of embryonic tissues or cells, intact embryos in culture, or free-living embryos are capable of distinguishing between mammalian developmental toxicants and nondevelopmental toxicants with an accuracy of > or = 80%. Despite this level of performance, there is still considerable reluctance among the scientific community to employ these assays for preliminary screening. In this paper, I review the theoretical basis for the predictiveness of these assays, outline the empirical data indicating their utility in screening toxicants, discuss the major limitations of in vitro assays and how they can be managed, and suggest applications for in vitro pre-screens. The embryo-derived assays should work because they continue to develop in vitro, and the underlying cellular and molecular processes driving this development are the same as those in the mammalian embryo in situ, and therefore, susceptible to the same insults. The assays do work, as specific mechanisms of developmental toxicity have been demonstrated in vitro, and because extensive validation studies have shown them to be highly concordant with traditional in vivo screens. The assays are inherently limited by the fact that they do not include all the levels of complexity of the maternal-embryonic unit; however, these limitations can be minimized by thoughtful assay selection, study design, and interpretation. Potential applications are suggested that complement but do not replace in vivo testing. Pre-screens will make product development more efficient and add to our knowledge about the developmental toxicity of previously untested compounds. In vivo screening would still be conducted on all classes of substances that are currently tested for developmental toxicity; however, fewer chemicals with high likelihood of being developmentally toxic, and therefore not appropriate for further commercial consideration, would be evaluated in these costly screens.

Deltour L, Ang HL, Duester G. **Ethanol inhibition of retinoic acid synthesis as a potential mechanism for fetal alcohol syndrome.** FASEB J 1996 Jul;10(9):1050-7.
Retinoic acid (RA) is known to act as a signaling molecule during embryonic development, but little is known about the regulation of RA synthesis from retinol. The rate-limiting step in RA synthesis is the oxidation of retinol, a reaction that can be catalyzed by alcohol dehydrogenase (ADH). Ethanol is also a substrate for ADH, and high levels of ethanol inhibit ADH-catalyzed retinol oxidation. This has prompted us to hypothesize that ethanol-induced defects observed in fetal alcohol syndrome involve ethanol inhibition of ADH-catalyzed RA synthesis. Here, we have examined the effect of ethanol on RA levels in cultured mouse embryos by using a bioassay. Treatment with 100 mM ethanol, but no 10 mM, led to a significant decrease in RA detection in 7.5-day-old embryos. Using whole-mount in situ hybridization, we detected mRNA for class IV ADH, but not ethanol-active cytochrome P450 2E1, in 7.5- and 8.5-day-old embryos, indicating that an ADH-linked pathway exists at these stages for metabolizing retinol and ethanol. Thus, the observed ethanol-induced reduction in RA may be caused by ethanol inhibition of retinol oxidation catalyzed by class IV ADH. In our postulated mechanism for fetal alcohol syndrome, this enzyme may well play a crucial role.

Depuydt CE, Mahmoud AM, Dhooge WS, Schoonjans FA, Comhaire FH. **Hormonal regulation of inhibin B secretion by immature rat sertoli cells in vitro: possible use as a bioassay for estrogen detection.** J Androl 1999;20(1):54-62.
The influences of follicle-stimulating hormone (FSH), gonadal steroids, and culture time were studied in

relation to inhibin B production by Sertoli cells of immature rats cultured in vitro. Sertoli cell-enriched cultures were established from 18-day-old rats and were maintained in medium supplemented with insulin, transferrin, and epidermal growth factor at 34 degrees C. A recently developed ELISA for the measurement of inhibin B was used to assess the effects of recombinant human FSH (rh FSH), testosterone (T), and estradiol (E2) on inhibin B production and accumulation in the culture media of Sertoli cell-enriched cultures and to optimize the cell culture system to serve as a bioassay for the detection and quantification of estrogens and estrogenlike substances. Prolonging the incubation time (24, 48, or 72 hours) of Sertoli cells with control medium without rh FSH, T, or E2 resulted in a time-dependent increase of inhibin B production. Incubation with rh FSH (1, 2.5, 5, or 10 U/L) caused a dose- and time-dependent increase of inhibin B production by Sertoli cells (but not by cultured Leydig cells), reaching a plateau at 5 U/L rh FSH. Addition of T in concentrations of 2.88, 5, or 50 ng/ml to medium without rh FSH and E2 significantly lowered the daily production rate of inhibin B ($P < 0.05$). In contrast, addition of E2 (0.01 and 0.1 ng/ml) caused a dose-responsive increase in inhibin B production after 24 and 48 hours. The relative increment of inhibin B production induced by E2 was maximal after 24 hours in the presence of 2.5 U/L rh FSH (acting synergistically) and in the absence of T. When these conditions are implemented, the Sertoli cell culture system may serve as a bioassay for estrogenic substances, and it may reflect the possibly harmful effect they may have on spermatogenesis.

Ekerfelt C, Matthiesen L, Berg G, Ernerudh J. **Paternal leukocytes selectively increase secretion of IL-4 in peripheral blood during normal pregnancies: demonstrated by a novel one-way MLC measuring cytokine secretion.** Am J Reprod Immunol 1997 Nov;38(5):320-6.
PROBLEM: It has been proposed that immune responses in normal pregnancy are Th2-like, thereby protecting the fetus and placenta from being rejected. Some studies have shown Th2-deviated systemic responses to different antigens and mitogens. The aim of this study was to demonstrate the specific T cell cytokine responses directed toward paternal histocompatibility leukocyte antigen (HLA), because this is the most prominent target for rejection of the feto-placental unit. METHOD OF STUDY: A novel one-way mixed leukocyte culture (MLC) combined with the detection of cytokine secretion with a sensitive ELISPOT assay was developed. Peripheral blood from 11 pregnant women was investigated with respect to allo-reactivity toward paternal leukocytes and pooled leukocytes from unrelated blood donors. This was done at three different occasions during pregnancy and 8 weeks after delivery. Nine age-matched non-pregnant women served as controls. RESULTS: In the second and third trimesters of pregnancy significantly larger numbers of IL-4-secreting cells (Th2) were induced by paternal leukocytes as compared to unrelated leukocytes. CONCLUSIONS: The findings indicate a selective immune deviation toward Th2, which may protect the fetus from rejection and thus may be an important homeostatic mechanism in normal pregnancies.

Ermilov A, Diamond MP, Sacco AG, Dozortsev DD. **Culture media and their components differ in their ability to scavenge reactive oxygen species in the plasmid relaxation assay.** Fertil Steril 1999;72 (1):154-7.
OBJECTIVE: To investigate the modulation of DNA-damaging effects of reactive oxygen species by media composition. DESIGN: In vitro study. SETTING: Academic medical center. PATIENT(S): None. INTERVENTION(S): None. MAIN OUTCOME MEASURE(S): Plasmid relaxation. RESULT(S): Ham's F-10 medium, 1% Percoll, superoxide dismutase (1, 10, or 100 IU), and synthetic serum

substitute did not affect DNA damage by reactive oxygen species and did not have any effect on plasmid DNA damage. Plasmid DNA damage was partially inhibited in the presence of P-1 and human tubal fluid media. Human serum albumin, phenol red, glucose, polyvinyl alcohol, polyvinylpyrrolidone, sucrose, and HEPES also were found to protect DNA from damage. CONCLUSION(S): In vitro fertilization media and their components vary widely in the way they affect DNA damage by reactive oxygen species.

Fein A, Carp H, Torchinsky A, Koifman M, Yacobovich R, Toder V. **Peri-implantation mouse embryos: an in vitro assay for assessing serum-associated embryotoxicity in women with reproductive disorders.** Reprod Toxicol 1998 Mar-Apr;12(2):155-9.

Finch RA, Gardner HS Jr, Bantle JA. **Frog embryo teratogenesis assay-Xenopus: a nonmammalian method for developmental toxicity assessment.** In: Salem, H, Editor. Animal Test Alternatives: Refinement, Reduction, Replacement. New York: M. Dekker; 1995. P. 297-313.
The frog embryo teratogenesis assay-Xenopus (FETAX) is a 96-hr, whole-embryo, nonmammalian developmental toxicity screening test that utilizes the embryos of the South African clawed frog Xenopus laevis. FETAX was first developed and standardized as a definitive assay for developmental toxicants by Dr. James Dumont and his co-workers at the Oak Ridge National Laboratory in 1983. The assay originally was developed to assess the potential developmental toxicity of complex mixtures derived from the synthetic fuels program at Oak Ridge National Laboratory. The assay is based on a large body of information generated by various researchers in studies on normal embryonic development in which the developing Xenopus embryo was used as the model system. The assay, as it is currently performed, is useful in screening for the potential developmental toxicity of both single chemicals such as pharmaceuticals or commodity chemicals, and complex chemical mixtures, such as environmental samples.

Fisher J, Mahle D, Bankston L, Greene R, Gearhart J. **Lactational transfer of volatile chemicals in breast milk.** Am Ind Hyg Assoc J 1997 Jun;58(6):425-31.
Lactational transfer of chemicals to nursing infants is a concern for occupational physicians when women who are breast-feeding return to the workplace. Some work environments, such as paint shops, have atmospheric contamination from volatile organic chemicals (VOCs). Very little is known about the extent of exposure a nursing infant may receive from the mother's occupational exposure. A physiologically based pharmacokinetic model was developed for a lactating woman to estimate the amount of chemical that a nursing infant ingests for a given nursing schedule and maternal occupational exposure. Human blood/air and milk/air partition coefficients (PCs) were determined for 19 VOCs. Milk/blood PC values were above 3 for carbon tetrachloride, methylchloroform, perchloroethylene, and 1,4-dioxane, while the remaining 16 chemicals had milk/blood PC values of less than 3. Other model parameters, such as solid tissue PC values, metabolic rate constants, blood flow rates, and tissue volumes were taken from the literature and incorporated into the lactation model. In a simulated exposure of a lactating woman to a threshold limit value concentration of an individual chemical, only perchloroethylene, bromochloroethane, and 1,4-dioxane exceeded the U.S. Environmental Protection Agency non-cancer drinking water ingestion rates for children. Very little data exists on the pharmacokinetics of lactational transfer of volatile organics. More data are needed before the significance of the nursing exposure pathway can be adequately ascertained. Physiologically based

pharmacokinetic models can play an important role in assessing lactational transfer of chemicals.

Forster C, Rucker M, Shakibaei M, Baumann-Wilschke I, Vormann J, Stahlmann R. **Effects of fluoroquinolones and magnesium deficiency in murine limb bud cultures.** Arch Toxicol 1998 Jun;72 (7):411-9.
Quinolone-induced arthropathy is probably caused by a lack of functionally available magnesium in immature joint cartilage. We used an in vitro assay to study the effects of fluoroquinolones on cartilage formation in mouse limb buds from 12-day-old mouse embryos in regular and in magnesium-deficient medium. Omission of magnesium from the medium had no adverse effect on the outcome of the culture: limb buds grew and differentiated well in regular and in magnesium-deficient Bigger's medium. Lack of calcium, however, severely impaired the development of the explants; this result was even more enhanced when both minerals (magnesium and calcium) were omitted. Electron microscopy revealed cell necrosis and deposition of electron-dense material in the vicinity of chondrocytes from limb buds after 6 days in a magnesium-free medium. A series of seven fluoroquinolones was tested at 30, 60, and 100 mg/l medium. At a concentration of 30 mg/l sparfloxacin only had a slight effect on limb development. At concentrations of 60 and 100 mg/l sparfloxacin, temafloxacin and ciprofloxacin impaired limb development in vitro concentration-dependently. The effects were enhanced in a magnesium-deficient medium (concentration of magnesium <10 micromol/l). Fleroxacin, lomefloxacin and ofloxacin impaired limb development only slightly; no significant differences were recognizable between the outcome in regular and in magnesium-deficient medium. Pefloxacin did not show any effect on limb development in both media. Using electron microscopy, very similar alterations as described above for the limbs cultured in magnesium-deficient medium were observed with ofloxacin at a concentration of 30 mg/l, which had no effect on the growth of the explants when evaluated macroscopically. The affinity of six fluoroquinolones to magnesium was determined by the use of a fluorescence assay. The affinity to magnesium correlated with the activity of the drugs in the limb bud assay. We conclude that fluoroquinolones have no effect on murine limb development in vitro at concentrations that are achieved under therapeutic conditions (peak concentrations approx. 1-5 mg/l in plasma). Effects at higher concentrations (60 and 100 mg/l) are slightly enhanced (factor 2) if the magnesium concentration in the medium is low. Macroscopically, limbs develop regularly in a magnesium-free medium, but ultrastructurally typical alterations are exhibited (e.g. cell necrosis and pericellular deposition of electron-dense material).

Fort DJ, Propst TL, Stover EL, Helgen JC, Levey RB, Gallagher K, Burkhart JG. **Effects of pond water, sediment, and sediment extracts from Minnesota and Vermont, USA, on early development and metamorphosis of Xenopus.** Environ Toxicol Chem 1999;18(10):2305-15.
BIOSIS COPYRIGHT: BIOL ABS. In recent studies, a high incidence of amphibian mortality and malformation has been reported in the field, suggesting that toxic and/or bioactive agents are present in the environment of the affected amphibians. This study provides evidence for this hypothesis, because it applies to several affected ponds in Minnesota and Vermont, USA. Three developmental bioassays were carried out on samples from three reference and three test sites in Minnesota and one reference and three test sites, in Vermo fects in vitro and in the field.

Fort DJ, Propst TL, Stover EL, Murray FJ, Strong PL. **Adverse effects from low dietary and environmental boron exposure on reproduction, development, and maturation in Xenopus laevis.** J

Trace Elem Exper Med 1999;12(3):175-85.
BIOSIS COPYRIGHT: BIOL ABS. In two separate 120-d depletion studies conducted previously, adult frogs (Xenopus laevis) fed a low boron diet (-B; 62 mug B/kg feed) for either 28 d or 12 d produced a greater proportion of necrotic eggs and fertilized embryos that abnormally gastrulated at a greater rate and were substantially less viable at 96 h of development when compared to embryos from adults administered a diet supplemented with boron (+B; 1850 mug B/kg feed). These studies showed that 28 d or 120 d of low boron markedl after just 28 d on the -B diet. Complete concentration-response curves were developed for boron in Xenopus using a 4 d embryo-larval developmental model (Frog Embryo Teratogenesis Assay: Xenopus (FETAX)), again documenting nutritionally essential, as well as toxic, concentration ranges. Deleterious developmental effects were induced in this assay at culture media concentrations of <0.3 muM B and >4980 muM B. Four-d Xenopus embryo-larval development within the range of 0.5-3320 muM B was shown t.

Fort DJ, Stover EL, Bantle JA, Rayburn JR, Hull MA, Finch RA, Burton DT, Turley SD, Dawson DA, Linder G, et al. **Phase III interlaboratory study of FETAX, Part 2: interlaboratory validation of an exogenous metabolic activation system for frog embryo teratogenesis assay--Xenopus (FETAX).** Drug Chem Toxicol 1998 Feb;21(1):1-14.
Interlaboratory validation of an exogenous metabolic activation system (MAS) developed for the alternative, short-term developmental toxicity bioassay, Frog Embryo Teratogenesis Assay-Xenopus (FETAX) was performed with cyclophosphamide and caffeine. Seven study groups within six separate laboratories participated in the study in which three definitive concentration-response experiments were performed with and without the MAS in a side-by-side format for each chemical. Since both chemicals had been previously tested in FETAX, the test concentrations were provided to each laboratory prior to testing. Interlaboratory coefficient of variation (CV) values for unactivated cyclophosphamide (no MAS) were 15%, 15%, 29%, and 25% for the 96-hr LC50, 96-hr EC50 (malformation), Minimum Concentration to Inhibit Growth (MCIG), and Teratogenic Index (TI) values, respectively. Addition of the MAS increased the CV values of each endpoint at least 3.9-fold. Interlaboratory CV values for unactivated caffeine were 31%, 18%, 31%, and 46% for the 96-hr LC50, 96-hr EC50 (malformation), MCIG, and TI values, respectively. Addition of the MAS decreased the CV values of each respective endpoint by at least 1.6-fold. Results indicated that bioactivated toxicants may be prone to greater variability in response amongst laboratories than compounds, which are detoxified. Even though more variability was noted with activated cyclophosphamide, results were within interlaboratory variation expected for other aquatic-based bioassays. Thus, results from these studies warrant the continued use and further refinement of FETAX for alternative developmental toxicity assessment.

Fort DJ, Stover EL, Propst T, Hull MA, Bantle JA. **Evaluation of the developmental toxicities of coumarin, 4-hydroxycoumarin, and 7-hydroxycoumarin using FETAX.** Drug Chem Toxicol 1998 Feb;21(1):15-26.
The developmental toxicities of coumarin and hydroxycoumarin metabolites were evaluated using FETAX. Young X. laevis embryos were exposed to coumarin, 4-hydroxycoumarin, and 7-hydroxycoumarin in each of two separate concentration-response experiments with and without an exogenous metabolic activation system (MAS) and/or inhibited MAS. The MAS was treated with carbon monoxide (CO), cimetidine (CIM), or ellipticine (ELL) to selectively modulate cytochrome P-

450 activity. The MAS was also treated with cyclohexene oxide (CHO) to selectively modulate epoxide hydrolase activity. Without the MAS or inhibited MAS, coumarin and 7-hydroxycoumarin were nearly equitoxic, whereas 4-hydroxycoumarin was nearly 2-fold less developmentally toxic than coumarin on an equimolar basis. Addition of the MAS and CIM-MAS increased the developmental toxicities of coumarin and, particularly, 4-hydroxycoumarin. Addition of the CHO-MAS greatly increased the developmental toxicity of coumarin and, especially, 4-hydroxycoumarin. Addition of the ELL- or CO-inhibited MAS did not increase the developmental toxicity of coumarin. However, addition of the intact MAS did not alter the developmental toxicity of 7-hydroxycoumarin. Results from these studies suggested that P-450; specifically ELL-inhibited P-450 (arylhydrocarbon hydroxylase) may have been responsible for increasing the developmental toxicity of coumarin. Furthermore, the increased toxicity of coumarin or 4-hydroxycoumarin following co-incubation with CHO-treated microsomes indicated that highly toxic epoxide intermediates may be produced from oxidative P-450 metabolism and that epoxide hydrolase may play a role in detoxification of the reactive intermediates.

Garman GD, Anderson SL, Cherr GN. **Developmental abnormalities and DNA-protein crosslinks in sea urchin embryos exposed to three metals.** Aquatic Toxicol 1997;39(3-4):247-65.
Two sublethal responses were used to investigate the effects of genotoxic metals on embryos of the purple sea urchin, Strongylocentrotus purpuratus. In addition to the standard measurement of developmental success, we used a novel genotoxic response, DNA-protein crosslink (DPC) induction, to assess the effects of embryo exposure to pentavalent arsenate (As), nickel (Ni) and hexavalent chromate (Cr). The procedure for sea urchin embryo DPC measurement was adapted from a mammalian cell assay using potassium-SDS precipitation and a DNA fluorochrome to quantify relative amounts of free and protein-bound DNA. Developmental abnormality and DPCs increased after a 48-h exposure to each of the three metals. Lowest observable effect concentrations (LOECs) for development were 0.011 mg/L As, 0.40 mg/L Ni, and 2.5 mg/L Cr. LOECs calculated for the DPC response to these same three metals were 0.023, 8.0, and 10.0 mg/L, respectively. DPCs were transiently high in transcription of the embryonic genome. By the gastrula and prism stages (subsequent to embryo gene transcription), there was a significant decrease in DPCs. Ni-exposed embryos exhibited the greatest magnitude of adverse effect in embryos exposed through the blastula stage, as compared to those exposed from blastula through late gastrula stage. We hypothesize that stage-sensitivity to Ni in sea urchin embryos may be related to the induction of persistent DPCs, and the prevention of normal transcription of the embryonic genome.

Gray MA, Niimi AJ, Metcalfe CD. **Factors affecting the development of testis-ova in medaka, Oryzias latipes, exposed to octylphenol.** Environ Toxicol Chem 1999;18(8):1835-42.
BIOSIS COPYRIGHT: BIOL ABS. There are many endocrine-disrupting chemicals in the environment that have the potential to alter the development of sexual characteristics in fish and wildlife. Little is known about the factors that influence the development of an intersex condition in fish. Japanese medaka (Oryzias latipes) were exposed to octylphenol (OP), a known estrogen agonist, during various life history stages to determine the factors that control induction of testis-ova, an intersex condition. In male medaka exposed t Exposures of adult male medaka to OP (200 and 300 mug/L) for either 18 or 36 d resulted in onlyone testis-ova in a male fish exposed for 36 d to the highest nominal concentration. In addition to testis-ova, male medaka exposed to OP developed testicular fibrosis. Overall, these data

indicate that prolonged exposure of male medaka to an estrogen agonist beginning around the period of gonadal differentiation is optimal for the development of testis-ova, but this intersex condition can be induced w.

Gronen S, Denslow N, Manning S, Barnes S, Barnes D, Brouwer M. **Serum vitellogenin levels and reproductive impairment of male Japanese Medaka (Oryzias latipes) exposed to 4-tert-octylphenol.** Environ Health Perspect 1999;107(5):385-90.
The induction of synthesis of the "female" yolk precursor protein vitellogenin (VTG) in male fish by estrogenic chemicals in the environment has been demonstrated in many recent reports. However, little is known about the organismal and biological significance of this phenomenon. To examine the relationship between VTG production in male fish and reproductive impairment, adult male medaka were exposed to 4-tert-octylphenol (OP), a known environmental estrogen, in concentrations ranging from 20 to 230 ppb for 21 days, under flow-through conditions. Following exposure, male fish were mated, in the absence of OP, with unexposed females. Breeding groups composed of exposed males and control females produced about 50% fewer eggs than control groups. VTG levels in serum of male fish increased with increasing OP exposure concentration and decreased after OP exposure was discontinued. Nevertheless, significant correlations ($p<0.01$) were observed between VTG levels in exposed male fish and 1) OP exposure concentrations, 2) percent of fertilized eggs, and 3) survival of embryos. OP-induced VTG synthesis and reproductive impairment appear to be closely linked phenomena. Histological examination indicated spermatogenesis in OP-exposed fish was inhibited, and some exposed fish had oocytes in their testes. Finally, OP caused a significant increase in the number of abnormally developing embryos, suggesting that OP may be genotoxic as well as estrogenic.

Henshel DS, Martin JW, Dewitt JC. **Brain asymmetry as a potential biomarker for developmental TCDD intoxication: a dose-response study.** Environ Health Perspect 1997 Jul;105(7):718-25.
Previous studies have indicated that in ovo exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds is correlated with the development of grossly asymmetric brains. This asymmetry is manifested as a difference between the two halves of the forebrain and the tecta. Previously, only wildlife species (heron, cormorant, and eagle) had been shown to manifest this response. In the wildlife studies, the frequency and degree of left-right interhemispheric differences had been correlated with the levels of polychlorinated dibenzo-p-dioxin toxic equivalency factors (TEFs) in eggs from the same nest (heron, cormorant). We studied the effect of in ovo exposure to TCDD on the brain throughout development in a sensitive laboratory model (chicken). Embryos from chicken eggs (Gallus gallus) injected with one of several doses of TCDD or vehicle control were sacrificed after 9, 11, 13, 15, 17, or 20 days of incubation, or incubated to hatch and then sacrificed either within 24 hr or at 3 weeks post-hatch. Measurements of both chicken embryo and hatchling brains indicated that 1) TCDD alone induced the brain asymmetry in developing chickens; 2) this brain asymmetry was similar to that observed in animals exposed in the wild to a mixture of TCDD-related contaminants; 3) there was a dose-related increase in both the frequency and severity of brain asymmetry observed at all ages measured; and 4) the asymmetry was measurable in embryonic brains at an age when the braincase was a thin, flexible layer (embryonic day 9), implying that the effect of TCDD was directly on the developing brain and not indirectly via an effect on the braincase.

Hoffman D$_{247}$ Melancon MJ, Klein PN, Rice CP, Eisemann JD, Hines RK, Spann JW, Pendleton GW.

Developmental toxicity of PCB 126 (3,3',4,4',5-pentachlorobiphenyl) in nestling American kestrels (Falco sparverius). Fundam Appl Toxicol 1996 Dec;34(2):188-200.
Planar PCB congeners are embryotoxic and teratogenic to birds including American kestrels. The developmental toxicity of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) was studied in the posthatching kestrel as a model for the eagle. Nestlings were dosed orally for 10 days with 5 microl/g body weight of corn oil (controls) or the planar PCB 126 at concentrations of 50, 250, or 1000 ng/g body weight. Dosing with 50 ng/g of PCB 126 resulted in a hepatic concentration of 156 ng/g wet weight, liver enlargement and mild coagulative necrosis, over 10-fold increases in hepatic microsomal ethoxyresorufin-O-dealkylase and benzyloxyresorufin-O-dealkylase, and approximately a 5-fold increase in methoxyresorufin-O-dealkylase. At this dose, mild to moderate lymphoid depletion of the spleen was apparent, as were decreased follicle size and content of the thyroid. At 250 ng/g, concentration of PCB 126 in the liver was 380 ng/g with increasing multifocal coagulative necrosis, decreased bone growth, decreased spleen weight with lymphocyte depletion of the spleen and bursa, and degenerative lesions of the thyroid. At 1000 ng/g, the liver concentration was 1098 ng/g, accompanied by decreased bursa weight, decreased hepatic thiol concentration, and increased plasma enzyme activities (ALT, AST, and LDH-L) in addition to the previous effects. Highly significant positive correlations were noted between liver concentrations of PCB 126 and the ratio of oxidized to reduced glutathone. These findings indicate that nestling kestrels are more susceptible to PCB 126 toxicity than adults, but less sensitive than embryos, and that planar PCBs are of potential hazard to nestling birds.

Hoffman LM, Kulyk WM. **Alcohol promotes in vitro chondrogenesis in embryonic facial mesenchyme.** Int J Dev Biol 1999;43(2):167-74.
Ethanol is a well-recognized teratogen in vertebrates that can perturb the development of the facial primordia and various other embryonic structures. However,the mechanisms underlying alcohol's effects on embryogenesis are currently unclear. Recent evidence suggests that the cranial neural crest, which forms the entire facial skeleton, may be a particularly sensitive target of ethanol teratogenicity. In the present study we have examined the influence of in vitro ethanol exposure on cartilage differentiation in micromass cultures of mesenchymal cells isolated from the various facial primordia (maxillary, mandibular, frontonasal, and hyoid processes) of the stage 24 chick embryo. In all four populations of facial mesenchyme, exposure to 1-1.5% ethanol promoted marked increases in Alcian blue-positive cartilage matrix formation, a rise in 35SO4 accumulation into matrix glycosaminoglycans, and enhanced expression of cartilage-characteristic type II collagen and aggrecan gene transcripts. In frontonasal and mandibular mesenchyme cultures, which undergo extensive spontaneous cartilage formation, ethanol treatment quantitatively elevated both matrix production and cartilage-specific gene transcript expression. In cultures of maxillary process and hyoid arch mesenchyme, which form little or no cartilage spontaneously, ethanol exposure induced the formation of chondrogenic cell aggregates and the appearance of aggrecan and type II collagen mRNAs. These actions were not restricted to ethanol, since tertiary butanol treatment also enhanced cartilage differentiation in facial mesenchyme cultures. Our findings demonstrate a potent stimulatory effect of alcohol on the differentiation of prechondrogenic mesenchyme of the facial primordia. Further analysis of this phenomenon might yield insight into the developmental mechanisms underlying the facial dysmorphologies associated with embryonic ethanol exposure.

Hutchinson TH, Pounds NA, Hampel M, Williams TD. **Impact of natural and synthetic steroids on the survival, development and reproduction of marine copepods (Tisbe battagliai).** Sci Total Environ 1999;233(1-3):167-79.
BIOSIS COPYRIGHT: BIOL ABS. Given recent reports suggesting that natural and synthetic steroids (namely, oestradiol, oestrone and ethynylestradiol) may be present in sewage effluent at levels which may impact on fish, it is pertinent to extend the ecological hazard evaluation for such substances to aquatic invertebrates. Studies have therefore been undertaken to address whether 17beta-oestradiol, oestrone and 17alpha-ethynylestradiol can inhibit survival, development or reproductive output in Tisbe battagliai (Crustacea, C ve control) and effects monitored in terms of survival, development and sex ratio after 10 days at 20 : 1~C. Adult males and females were then paired and exposures continued to investigate effects on reproductive output (21 days total exposure). In summary, the lowest 21 day No Observed Effect Concentrations based on these life-cycle parameters were: 20-hydroxyecdysone: 8.7 mug l-1; oestrone: : 100 mug l-1; 17beta-oestradiol: : 100 mug l-1; and 17alpha-ethynylestradiol: : 100 mug l-1 (all based.

Islinger M, Pawlowski S, Hollert H, Volkl A, Braunbeck T. **Measurement of vitellogenin-mRNA expression in primary cultures of rainbow trout hepatocytes in a non-radioactive dot blot/RNAse protection-assay.** Sci Total Environ 1999;233(1-3):109-22.
The induction of vitellogenin synthesis both in vivo and in vitro has proven to be a reliable biomarker for assessing the estrogenic activity of individual substances and the more complex effluents of sewage treatment plants. However, due to the requirement of radioactively labelled nucleotides, the measurement of vitellogenin-mRNA has not been widely used in routine testing--even though this technique promises elevated sensitivity. In order to develop a practicable, reliable and cost-effective bioassay suitable for routine testing, a combined dot-blot/RNAse protection assay, utilising digoxigenin-labelled cRNA transcripts of plasmid psg5Vg1.1 was used for the quantification of vitellogenin-mRNA in isolated rainbow trout (Oncorhynchus mykiss) hepatocytes. By re-cloning the Vg1.1 insert into a pGemZf7(-)-vector, the sense-transcript of Vg1.1 was utilized as a standard for the quantification of vitellogenin-mRNA concentrations. Male rainbow trout hepatocytes were cultured as monolayers in pure M199 medium. The addition of serum supplements did not result in increased expression of vitellogenin-mRNA following 17 beta-estradiol administration. This indicates that for this assay no supplementation of the culture medium is necessary. After addition of 17 beta-estradiol, hepatocytes exhibited an exponential time-dependent expression of vitellogenin-mRNA over a period of 144 h. The dot blot system was sufficiently sensitive to detect vitellogenin-mRNA following addition of 1 microM 17 beta-estradiol after 6 h of incubation. However, the amount of vitellogenin-mRNA expressed was found to be a function of both incubation time and inducer concentration. Prolonged incubation times were therefore required to enhance the sensitivity of the system. After a 96-h incubation, detection limits for 17 beta-estradiol were between 100 pM and 1 nM. Vitellogenin-mRNA could not be detected in untreated hepatocytes. The vitellogenin-mRNA dot blot/RNAse protection assay was further used as a tool for assessing the estrogenic potential of the xenoestrogens nonylphenol and bisphenol A, which exhibited estrogenic activities approximately 2000-fold less than the natural inducer 17 beta-estradiol. The vitellogenin-mRNA response to 17 alpha-ethinylestradiol reached maximum efficacy down to the lowest tested concentration of 10(-9) M. The assay also successfully identified estrogenic activity in selected waste water samples.

Jackson GM, Edwin SS, Varner MW, Casal D, Mitchell MD. **Regulation of fetal fibronectin production in human amnion cells.** J Soc Gynecol Investig 1996 Mar-Apr;3(2):85-8.
OBJECTIVE: We evaluated the role of human amnion in the production of fetal fibronectin and assessed the regulation of fetal fibronectin production by inflammatory products and cytokines. METHODS: Human amnion cells were grown in culture. At confluence, the cell were incubated with and without lipopolysaccharide, interleukin-1 beta, tumor necrosis factor-alpha, and interleukin-6. Fetal fibronectin production was measured in the supernatant fluid using an enzyme-linked immunosorbent assay technique. RESULTS: Unstimulated amnion cells produced fetal fibronectin, and production was increased by lipopolysaccharide, interleukin-1 beta, tumor necrosis factor-alpha, and interleukin-6. CONCLUSION: Human amnion cells in vitro produce fetal fibronectin in substantial quantities. This production is stimulated by inflammatory products and mediators that are considered to be important in the initiation of some cases of preterm labor.

Janz DM, Bellward GD. **In ovo 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure in three avian species. 2. Effects on estrogen receptor and plasma sex steroid hormones during the perinatal period.** Toxicol Appl Pharmacol 1996 Aug;139(2):292-300.
As opposed to mammals, the heterogametic sex in birds is female, and sexual differentiation of the central nervous system away from the intrinsic male pattern is dependent on ovarian estrogen secretions during the perinatal period. The contamination of aquatic systems with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds has been suggested to be responsible for decreased reproductive success in certain wild fish-eating bird populations. Since TCDD has been shown to alter estrogenic status in laboratory animals, we determined the effects of in ovo TCDD exposure on hepatic estrogen receptor (ER) concentrations and affinities, and plasma estradiol concentrations during the perinatal period in the domestic chicken (Gallus gallus), domestic pigeon (Columba livia), and great blue heron (Ardea herodias). Plasma testosterone levels were also determined in herons as an indication of androgenic status. [3H]TCDD was injected into the air cell of chicken eggs on Embryonic Day 4.5 (0.1 microgram/kg egg), pigeon eggs on Embryonic Day 3.5 (1 microgram/kg egg) and Embryonic Day 14 (3 micrograms/kg egg), and heron eggs at approximately Embryonic Day 13 (2 micrograms/kg egg). Chickens were euthanized on Embryonic Days 17 and 19, hatch, and Days 2 and 4 after hatch. Pigeons and herons were either euthanized at hatch or fed an uncontaminated diet for 7 days prior to termination. Between 5 and 10% of the injected [3H]TCDD dose was measured in the liver of hatchlings. There was no effect of in ovo TCDD exposure on hepatic ER levels or plasma estradiol concentrations in female chickens and pigeons exposed early in incubation. In female pigeons exposed during the latter third part of incubation to a TCDD dose that would cause high embryo lethality if injected early in incubation, hepatic ER concentrations were elevated ($p < 0.001$) and plasma estradiol concentrations were decreased ($p < 0.01$) at hatch. There was no effect of TCDD exposure on plasma estradiol levels in male pigeons. In herons, TCDD exposure had no effect on hepatic ER levels or plasma estradiol and testosterone concentrations at either time point. We conclude that in chicken, pigeon, and great blue heron hatchlings exposed early in incubation to low doses of TCDD, hepatic ER levels and plasma estradiol concentrations are not biomarkers of toxicity.

Jenkins PJ, Cross TA, Perry LA, Medbak SA, Besser GM, Clark AJ. **The influence of plasma on basal and ACTH$_{1-24}$-stimulated in vitro adrenocortical steroidogenesis.** J Endocrinol 1999;162(1):155-61.

Early descriptions of in vitro ACTH bioassays all emphasised the need to use extracted plasma samples due to interference by an unidentified component. The aim of these studies was to elucidate the effects of whole plasma on ACTH steroidogenic activity in vitro and to identify the responsible factor. A sensitive in vitro dispersed bovine adrenocortical cell bioassay was established. The addition of 10% ACTH-depleted human pooled plasma to the incubation media resulted in basal steroidogenesis equivalent to that achieved with 10(-9) M ACTH1-24 and potentiated the steroidogenic activity of 10(-9) M ACTH1-24 by 7.8-fold. This potentiation was dependent on the concentration of both ACTH and plasma in the media, but did not result from the mitogenic effect of plasma. A pituitary source was excluded and the potentiating activity was not extractable by Vycor glass. Column chromatography demonstrated two peaks of activity corresponding to molecular weights of 650 and 220x10(3) Da. These peaks did not correspond to the plasma binding of 125I-ACTH which resulted from non-specific binding to albumin. Lipoprotein-deficient serum had no effect on either basal or ACTH-stimulated steroidogenesis, but both were restored by the addition of purified lipoproteins. However, novel findings demonstrated a differential effect of low (LDL) and high (HDL) density lipoproteins on basal and ACTH-stimulated steroid production; thus, LDL exerted a greater effect on the former, whilst HDL potentiated the steroidogenic activity of added ACTH more than LDL. The addition of the lipoproteins to lipoprotein-deficient serum restored its basal and ACTH potentiating effects, the cholesterol concentrations of the chromatographic fractions exactly paralleling their ACTH potentiating effect. These findings suggest that not only are lipoproteins the plasma factor(s) which potentiates ACTH steroidogenic activity in in vitro bioassays, but also that they exert differential effects on basal and ACTH-stimulated steroid production.

Kim Y, Cooper KR. **Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyls (PCBs) in the embryos and newly hatched larvae of the Japanese medaka (Oryzias latipes).** Chemosphere 1999;39(3):527-38.
BIOSIS COPYRIGHT: BIOL ABS. Studies were undertaken to evaluate the toxicities of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and five PCB congeners using the Japanese medaka embryo-larval assays. The embryos and newly hatched larvae exposed to TCDD and two non-ortho PCB congeners; 77 and 126, showed a similar type and sequence of lesions. The toxic effects in the cardiovascular system were the first to appear. The typical lesions observed were multifocal hemorrhages, pericardial and yolk sac edema, craniofacial malformatio se any toxic effects on the embryos exposed up to 2000 ng/ml. However, all three PCB congeners (105, 138 and 153) caused an inhibition of swim bladder inflation in the newly hatched larvae. The inhibition of swim bladder inflation was the most sensitive end point measured, and it is suggested that the inhibition of SBI may be mediated by an unknown mechanism with/without an AhR activation.

Klug S, Merker HJ, Jsackh R. **Potency of monomethyl-, dimethylformamide and some of their metabolites to induce abnormal development in a limb bud organ culture.** Toxicol In Vitro 1998 Apr;12(2):123-32.
DMF, NMF and their major metabolites were investigated for their developmental toxicity in the mouse limb bud assay. We found that neither DMF, NMF nor the predominant urinary metabolite HMFF exhibited developmental activity. In contrast, all metabolites resulting from the glutathione binding pathway, SMG, SMC and AMCC showed potent developmental activity. Under the chosen exposure

conditions, the developmental toxicity of DMF in different species appears to be related to the magnitude of glutathione binding. The results further show the value of using an in vitro system which is incapable of metabolic transformation of exogenous compounds for the identification of ultimate teratogenic species.

Kortenkamp A, Altenburger R. **Approaches to assessing combination effects of oestrogenic environmental pollutants.** Sci Total Environ 1999;233(1-3):131-40.
Concerns about possible combination effects of environmental chemicals with estrogenic activity have motivated the search for synergisms between such agents. However, much published work has taken no account of the concepts and methods for analysing combination effects, which were developed in toxicology and pharmacology. In the present communication, we draw attention to conceptual frameworks relevant for a sound analysis of the effects of mixtures of oestrogenic compounds. A model calculation is presented demonstrating that it is conceivable that weakly oestrogenic compounds may be able to act together to produce significant effects, even when they are present at concentrations below their individual effect thresholds. Our results suggest that it may not be necessary to invoke synergisms in order to explain the discrepancy between the high concentrations of these agents required to produce effects in in vitro assay systems and their low concentrations in the environment.

Koundakjian EJ, Bournias-Vardiabasis N, Haggren W, Adey WR, Phillips JL. **Exposure of Drosophila melanogaster embryonic cell cultures to 60-Hz sinusoidal magnetic fields: expression of heat shock proteins 23 and 70.** In Vitro Toxicol 1996 Fall;9(3):281-90.
Whether or not exposure to low-level extremely low frequency magnetic fields (MFs) poses hazards to human health and development remains an unresolved issue. Central to the problem is the lack of detailed and plausible biological mechanisms to explain how such exposures could lead to human pathology. Several investigators have reported the induction of a stress response at physiological, biochemical, and molecular levels in various biological systems exposed to MFs. Consequently, an in vitro teratogenesis (developmental toxicity) assay employing embryonic Drosophila cells has been used to determine whether or not exposure to a 60-Hz MF of either 10 or 100 uT (i.e., 100 or 1000 mGauss, respectively) could itself induce expression of heat shock proteins (hsp) 23 and 70, or whether such an exposure could potentiate heat shock protein expression induced by a chemical teratogen (developmental toxicant). Results demonstrated that (1) MF exposure of undifferentiated or differentiated cells at either field intensity did not alter expression of hsp23 or hsp70 from that observed in unexposed control cell cultures; (2) MF exposure (16 h) of cell cultures with three chemical teratogens (retinoic acid, hydroxyurea, and cadmium) did not alter expression of hsp23 or hsp70 from that observed in unexposed control cultures. These data do not support the thesis that MF exposure serves as a biological stressor.

Kramer VJ, Giesy JP. **Specific binding of hydroxylated polychlorinated biphenyl metabolites and other substances to bovine calf uterine estrogen receptor: structure-binding relationships.** Sci Total Environ 1999;233(1-3):141-61.
The objectives of this research were: (1) to survey a wide variety of structurally diverse (and mostly chlorinated) aromatic chemicals for specific binding to the calf uterine estrogen receptor; (2) to develop a quantitative structure-binding relationship (QSBR) for hydroxylated polychlorinated biphenyls (OH-PCBs). This report specifically includes data on substances that did not exhibit specific binding to ER

thereby exploring the structural requirements for specific binding to the estrogen receptor. Although several other QSBRs for OH-PCBs have been reported, this study presents data on a larger, environmentally relevant set of OH-PCBs than previously reported. Fifty three chemicals were tested for the ability to bind specifically to calf uterine estrogen receptor. All but three OH-PCBs bound specifically to calf uterine ER. For DDT compounds, receptor binding affinity followed the pattern: o,p'-DDT > o,p'-DDE > o,p'-DDD (Not active). Also exhibiting measurable affinity were 17 beta-estradiol (a positive control and the native ligand of the estrogen receptor), 2,4,6-trichlorobiphenyl and 4-chloro-2-isopropyl-5-methylphenol. Substances that did not bind to calf uterine estrogen receptor comprised several individual PCB congeners, chlorinated naphthalenes and naphthalenols, chlorinated bibenzyls, chlorinated phenols, and 9-chloro-retene. For 25 hydroxylated PCBs, a five parameter QSBR was developed using multiple linear regression and selection of the most parsimonius model from a total of seven molecular modeling parameters examined. The QSBR model predicted the ER binding log (IC50) to within one log unit.

Kuwagata M, Takashima H, Nagao T. **A comparison of the in vivo and in vitro response of rat embryos to 5-fluorouracil.** J Vet Med Sci 1998 Jan;60(1):93-9.
This study serves to further define the capabilities of the whole embryo culture system using the well-known teratogen, 5-fluorouracil (5-FU), an antineoplastic agent. An initial in vivo study was performed whereby pregnant rats were injected intraperitoneally with 10-30 mg/kg 5-FU on day 9 of gestation. On day 20 of gestation, the effects of this drug on the growth and development of embryos were evaluated. The number of externally malformed fetuses increased in a dose-related manner, and the most common defect was micro-/anophthalmos in fetuses of dams treated with 5-FU. Growth retardation was also noted in the 5-FU treated groups. An in vitro study was performed in which drug concentrations were varied (0.15-0.30 microg/ml). Externally abnormal embryos were observed in whole embryo culture system from embryonic day 9 to 11. The most common defect was hypoplastic optic vesicles. In the whole embryo culture system, crown-rump length, somite number, protein contents, and morphological score were decreased in a dose-dependent fashion. Finally, histological evaluation and observation of the pattern of cell death of the optic vesicle of 11-day-old embryos in in vivo and in vitro were performed. These parameters revealed no differences in response between in vivo and in vitro embryos treated with 5-FU, suggesting that the whole embryo culture system was an appropriate model for developmental toxicity studies of 5-FU.

Laws SC, Carey SA, Kelce WR, Cooper RL, Gray LE Jr. **Vinclozolin does not alter progesterone receptor (PR) function in vivo despite inhibition of PR binding by its metabolites in vitro.** Toxicology 1996 Sep 2;112(3):173-82.
Vinclozolin, a dicarboximide fungicide, alters morphological sex differentiation in male rats following perinatal exposure. The occurrence of these abnormalities correlates with the in vivo formation of two antiandrogenic metabolites of vinclozolin, (i.e. 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (Mt) and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2)), which are potent inhibitors of rat androgen receptor binding. As steroid hormone receptors exhibit promiscuity in their ability to bind different ligands, the present study evaluated the ability of these vinclozolin metabolites to bind to the estrogen (ER) and progesterone (PR) receptors in vitro, and to alter ER and PR function following in vivo exposure. To this end, in vitro ligand binding assays demonstrated that both M1 and

M2 can compete with endogenous ligand for binding to the PR (Ki = 400 and 60 microM, respectively). In contrast, neither metabolite exhibited the ability to bind ER. Subsequent in vivo studies to evaluate the potential of vinclozolin to alter ER or PR function demonstrate that, (1) the estrogen-dependent increases in uterine weight and PR induction were not altered by vinclozolin; (2) the distribution of nuclear and cytosolic PR was not altered following short-term vinclozolin exposure; and (3) vinclozolin did not disrupt ovulation in cycling female rats. These studies indicate that although vinclozolin metabolites can compete for binding to the PR in vitro, concentrations of these metabolites do not reach sufficient levels to disrupt female reproductive function following short-term in vivo exposure to vinclozolin. In addition, these studies demonstrate the importance of correlating in vitro receptor binding data with in vivo studies in order to understand the physiological consequences of exposure to environmental toxicants.

Legler J, Van Den Brink CE, Brouwer A, Murk AJ, Van Der Saag PT, Vethaak AD, Van Der Burg B. **Development of a stably transfected estrogen receptor-mediated luciferase reporter gene assay in the human T47D breast cancer cell line.** Toxicol Sci 1999;48(1):55-66.
Development of an estrogen receptor-mediated, chemical-activated luciferase reporter gene-expression (ER-CALUX) assay was attempted by stable transfection of luciferase reporter genes in a number of cell lines. Stable transfection of the chimeric Gal4 estrogen receptor and luciferase gene constructs in MCF-7 breast cancer and Hepa.1c1c7 mouse hepatoma cell lines, as well as transfection of a newly constructed luciferase reporter gene pEREtata-Luc in the ECC-1 human endometrial cell line, resulted in constitutive, non-estradiol-inducible clones. Stable transfection of pEREtata-Luc in the T47D breast cancer cell line, however, resulted in an extremely sensitive, highly responsive cell line. Following a 24-h exposure to estradiol (E2), stably transfected T47D.Luc cells demonstrated a detection limit of 0.5 pM, an EC50 of 6 pM, and a maximum induction of 100-fold relative to solvent controls. No clear reduction in responsiveness has been found over extended culture periods (50 passages). Anti-estrogens ICI 182,780, TCDD, and tamoxifen inhibited the estradiol-mediated luciferase induction. Genistein, nonylphenol, and o,p'DDT were the most potent (pseudo-)estrogens tested in this system (EC50 100, 260, and 660 nM, respectively). Determination of interactive effects of the (pseudo-)estrogens nonylphenol, o,p'DDT, chlordane, endosulfan, dieldrin, and methoxychlor revealed that, in combination with 3 pM E2, (pseudo-)estrogens were additive. Slightly more than additive effects (less than 2-fold) were found for combinations of dieldrin and endosulfan tested in the range of 3 to 6 microM. At these concentrations, the combination of endosulfan and chlordane demonstrated additive interaction. The ER-CALUX assay with T47D cells can provide a sensitive, responsive, and rapid in vitro system to detect and measure substances with potential (anti-)estrogenic activity.

Li J, Wei Y, Wagner TE. **In vitro endothelial differentiation of long-term cultured murine embryonic yolk sac cells induced by matrigel.** Stem Cells 1999;17(2):72-81.
The yolk sac of an early mammalian embryo contains progenitors of hematopoietic cells and vascular endothelial cells. We established a cell line, YS4, from murine embryonic yolk sac 10 years ago. The line has been successfully cultured since then. To determine whether these long-term cultured yolk sac cells still have the potential to differentiate into endothelial cells, an in vitro model of yolk sac cell differentiation into tubeforming endothelial cells was established in the present study by culturing the yolk sac cells on basement membrane proteins (Matrigel). The results indicate that upon plating onto

Matrigel, YS4 cells attach quickly, align in tandem, and form a complete network of capillary structures within 12 h. By using antibodies against the known components of Matrigel in a tube formation inhibition assay, we found that extracellular matrix proteins such as laminin, collagen IV, vitronectin, and fibronectin are the most important components in the Matrigel which induce the yolk sac cells to undergo endothelial differentiation. New basement membrane proteins are also required for the endothelial differentiation process, as indicated by the fact that base membrane protein synthesis inhibitor, D609, can block the differentiation process. Furthermore, our experiments revealed the involvement of several signal transduction pathways, such as protein kinase A, C and protein tyrosine kinase in this differentiation process.

Livezey GT, Smith CV. **The quantified EEG frequency profile as a biomarker of altered brain development.** Neurotoxicol Teratol 1998 May/Jun;20(3):369-70.
Our animal models of prenatal exposure to therapeutic doses of diazepam, lorazepam, phenobarbital and fluoxetine have demonstrated permanently altered behavioral and EEG profiles in the absence of gross morphological markers. Our studies and others have confirmed drug-specific receptor deficits in the adult offspring after prenatal exposure. The power spectral analysis of the EEG provides drug, dose and gender specific frequency profiles that are "signatures" of the correlated behavioral and learning deficits. The EEG spectra and behavioral correlates appear as mirror images of the profiles seen with acute drug challenge of a drug naive subject. To test the clinical implications of these data, we are collecting neonatal EEGs and applying similar frequency analysis techniques. Telemetry recording caps allow for continuous monitoring without influencing the experience for mother or child. We incorporate a 24 hour scan to produce a measure of each subjects "functional repertoire" which can be normalized for comparison of subjects. We are validating this approach as a routine fetal and neonatal diagnostic tool. The data has confirmed normative trends in neonatal EEG profiles. Potential applications include nearly all prenatal insults to brain development.

Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, Wainscoat JS, Johnson PJ, Chang AM, Hjelm NM. **Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis.** Am J Hum Genet 1998 Apr;62(4):768-75.
We have developed a real-time quantitative PCR assay to measure the concentration of fetal DNA in maternal plasma and serum. Our results show that fetal DNA is present in high concentrations in maternal plasma, reaching a mean of 25.4 genome equivalents/ml (range 3.3-69. 4) in early pregnancy and 292.2 genome equivalents/ml (range 76. 9-769) in late pregnancy. These concentrations correspond to 3.4% (range 0.39%-11.9%) and 6.2% (range 2.33%-11.4%) of the total plasma DNA in early and late pregnancy, respectively. Sequential follow-up study of women who conceived by in vitro fertilization shows that fetal DNA can be detected in maternal serum as early as the 7th wk of gestation and that it then increases in concentration as pregnancy progresses. These data suggest that fetal DNA can be readily detected in maternal plasma and serum and may be a valuable source of material for noninvasive prenatal diagnosis.

Mantovani A, Stazi AV, Macri C, Maranghi F, Ricciardi C. **Problems in testing and risk assessment of endocrine disrupting chemicals with regard to developmental toxicology.** Chemosphere 1999;39 (8):1293-300.
Endocrine disrupting chemicals (EDCs) may affect mammalian development either indirectly (by

impairing implantation, placental development, lactation, etc.) or directly, altering the maturation of target tissues. Current regulatory tests for reproductive/developmental toxicity should be carefully evaluated with regard to risk assessment of EDCs, considering hazard identification (are relevant endpoints being assessed?) and dose-response assessment (are sensitive NOEL/dose-response curves being provided?). Many in vitro and in vivo assays for sex steroid disruption are available; provided that the metabolic capacities of the assays are defined, they could be integrated in a sensitive battery for early detection of steroid-disrupting potentials. The screening battery should address further regulatory in vivo tests (e.g. what specific parameters have to be investigated). As regards dose-response, qualitative differences may be observed between lower and higher exposures, showing primary hormone-related effects and frank embryotoxicity, respectively. Other problems concern (a) the identification of critical developmental windows, according to hormone concentrations and/or receptor levels in the developing target tissues; (b) the potential for interactions between chemicals with common mechanism/target (e.g. xenoestrogens); (c) most important, besides sex steroids more attention should be given to other mechanisms of endocrine disruption, e.g., thyroid effects, which can be highly relevant to prenatal and postnatal development.

Massaad C, Barouki R. **An assay for the detection of xenoestrogens based on a promoter containing overlapping EREs.** Environ Health Perspect 1999;107(7):563-6.
BIOSIS COPYRIGHT: BIOL ABS. Xenoestrogens could be implicated in the decrease of male fertility and in the increased incidence of testicular and breast cancers in humans. To predict their deleterious effects, various in vivo or in vitro tests have been proposed to assay the xenoestrogenic activity. We have designed an assay for the detection of xenoestrogens based on a novel estrogen responsive unit formed by two overlapping estrogen response elements (overEREs). This construct is able to mediate a synergistic activation o ereas the overERE unit allowed us to detect a significant estrogenic activity of endosulfan at a lower concentration (10-6 M). Some compounds did not exhibit any estrogenic activity when tested with a classical ERE, whereas they were potent xenoestrogens when the overERE was used (i.e., Betanal). The assays we have developed are very sensitive and can be performed quickly. Moreover, because the promoter that we used contains only an overlapping ERE as a regulatory unit, the interference of the t.

Meibohm B, Derendorf H, Mollmann H, Frohlich P, Tromm A, Wagner M, Homrighausen S, Krieg M, Hochhaus G. **Mechanism-based PK/PD model for the lymphocytopenia induced by endogenous and exogenous corticosteroids.** Int J Clin Pharmacol Ther 1999;37(8):367-76.
OBJECTIVE: Lymphocytopenia is a sensitive surrogate marker for the immunological effects of corticosteroids. This pharmacokinetic/pharmacodynamic (PK/PD) study investigated whether the circadian variation of blood lymphocytes observed after placebo is secondary to the circadian rhythm of endogenous cortisol, and developed based on this relationship an improved PK/PD model for a more sensitive description of the effect of low-dose corticosteroid therapy on blood lymphocytes considering the net activity of the exogenous corticosteroid budesonide and endogenous cortisol. METHODS: In an open, parallel study design, 3 mg oral budesonide or placebo were given at 8.00 a.m., 4.00 p.m. and midnight to two groups of 12 volunteers. Lymphocyte counts and serum concentrations of budesonide and cortisol were monitored for 24 hours. A mechanism-based PK/PD model which considered the non-linear protein binding of cortisol and the budesonide-induced cortisol suppression was employed to

relate changes in blood lymphocytes to free cortisol levels after placebo and to the net activity of free budesonide and free endogenous cortisol after active treatment. RESULTS: The circadian rhythm of blood lymphocytes observed after placebo could inversely be related to the circadian rhythm of serum cortisol. After budesonide administration, lymphocyte counts could accurately be linked to the net activity of budesonide and endogenous cortisol. The resulting EC50 values for the effect of budesonide on cortisol, budesonide on lymphocytes and cortisol on lymphocytes were 0.063 +/- 0.034, 0.22 +/- 0.13 and 26.3 +/- 15.0 ng/ml (placebo group 15.4 +/- 3.4 ng/ml), respectively. CONCLUSIONS: The presented mechanism-based PK/PD model suggests that blood lymphocytes are under physiological control of cortisol. It further indicates that endogenous and exogenous corticosteroids and their pharmacological interaction need to be considered for modeling the effects of low doses of exogenous corticosteroids on the immune system.

Menditto A, Turrio-Baldassarri L. **Environmental and biological monitoring of endocrine disrupting chemicals.** Chemosphere 1999;39(8):1301-7.
Trends toward an increase of adverse health effects on reproductive organs have been reviewed. An urgent need has been recognised to establish validated in vivo and in vitro screening assays to test for hormonal activities of chemicals. Relevant existing OECD guidelines have been reviewed and enhancements of some of these have been identified, mainly to test for estrogenic and androgenic activity of chemicals. The problems connected to monitoring activities are outlined, particularly for ambient and biological monitoring. Indeed, the problem of assessing human exposure to endocrine disrupting chemicals through environmental chemical analysis tends to a very high level of complexity. This has been illustrated through the example of one single subclass of endocrine disrupting compounds (EDCs), the organohalogen compounds. Valid biological markers are also needed to be effectively used in epidemiological studies and risk assessment. A multidisciplinary approach and the collaboration among experts in the field of clinical biochemistry, toxicology, and epidemiology is required.

Miller MR, Wentz E, Ong S. **Acetaminophen alters estrogenic responses in vitro: inhibition of estrogen-dependent vitellogenin production in trout liver cells.** Toxicol Sci 1999;48(1):30-7.
The purpose of this study was to determine if acetaminophen altered estrogen-dependent vitellogenin production in isolated trout liver cells. Estrogen-induced vitellogenesis was studied in liver cells isolated from male trout and cultured in defined medium; vitellogenin secreted into culture medium was quantitated using immunological procedures. Vitellogenin production was absolutely dependent on the addition of estradiol (10(-6) M) to liver cells from male trout. Acetaminophen produced a dose-dependent inhibition of vitellogenin production; approximately 50% inhibition was achieved with 0.05 mM acetaminophen, while 0.3 mM acetaminophen inhibited secreted vitellogenin to undetectable levels. In contrast, these concentrations of acetaminophen (< or = 1 mM) did not significantly alter the production of secreted albumin, determined immunologically, or cause detectable toxicity. Higher doses of acetaminophen were toxic, but did not induce DNA fragmentation in the trout liver cells. Acetaminophen reduction of estradiol-induced vitellogenin production was accompanied by a dose-dependent decrease in vitellogenin mRNA, indicating acetaminophen inhibited a step prior to, or during, formation of vitellogenin mRNA. Estrogen receptor-binding assays demonstrated that acetaminophen did not reduce binding of [3H]-estradiol to trout liver estrogen receptor. In addition, catabolism of estradiol to water-soluble metabolites was not significantly altered by acetaminophen. These studies

indicate that non-toxic concentrations of acetaminophen specifically inhibit estrogen-dependent vitellogenin synthesis and suggest that this commonly used drug may alter estrogen-regulated processes.

Miranda S, Malan Borel I, Margni R. **Altered modulation of the in vitro antibody synthesis by placental factors from the CBA/J x DBA/2 abortion-prone mating combination.** Am J Reprod Immunol 1998 May;39(5):341-9.
PROBLEM: The in vitro immunomodulating effect of placental culture supernatants (PSs) obtained from two H-2k x H-2d allogeneic crossbreedings, the CBA/J x DBA/2 abortion-prone mating combination, and the reproductively normal pregnancy CBA/J x BALB/c crossbreeding were compared, and the influence of previous deliveries was evaluated. The behavior of placentae obtained from CBA/J females with two previous pregnancies by BALB/c males was also investigated. METHOD OF STUDY: Supernatants of cultures of murine placentae were added to a mouse immunoglobulin (Ig) G1 hybridoma culture which produced anti-dinitrophenol (anti-DNP) antibodies. The quantity of monoclonal antibody produced, the nature of these antibodies, and the proliferation of the hybridoma cells were studied. RESULTS: CBA/J x DBA/2 placental factors obtained from multiparous females induced a diminished asymmetric IgG antibody production without varying the quantity of antibody produced. In contrast, PSs obtained from the nonresorption-prone CBA/J x BALB/c mating combination with the same number of previous deliveries enhanced the production of both symmetric and asymmetric anti-DNP molecules and also increased the proportion of asymmetric blocking monoclonal antibodies (mAbs) synthesized by the hybridoma. Both of the PSs analyzed had induced similar inhibition of 3H-thymidine uptake. PSs obtained from the abortion-prone mating combination whose CBA/J females had two previous pregnancies by BALB/c males showed similar immunomodulating effects to those observed using multiparous CBA/J x BALB/c placentae. CONCLUSIONS: We propose that the placenta produces soluble factors that participate in the regulation of antibody synthesis by the mother during gestation. Such a placental immunomodulating effect appears to be altered in the CBA/J x DBA/2 abortion-prone mating combination and could be corrected by previous pregnancies by BALB/c males. These observations suggest that placental factors would be relevant to the protection of the fetus and might play an important role in the immune equilibrium between mother and fetus. Asymmetric antibody production as a Th2 responsiveness was also discussed.

Mitchell JJ, Paiva M, Heaton MB. **Vitamin E and beta-carotene protect against ethanol combined with ischemia in an embryonic rat hippocampal culture model of fetal alcohol syndrome.** Neurosci Lett 1999;263(2-3):189-92.
Neurodevelopmental damage can occur as a result of in utero exposure to alcohol. Oxidative stress processes are one of many proposed mechanisms thought to contribute to nervous system dysfunction characterized in fetal alcohol syndrome (FAS). Therefore, this study examined neuroprotective effects of antioxidant supplementation during ethanol (EtOH) treatment (0, 200, 400, 800 or 1600 mg/dl) combined with concomitants of EtOH exposure: acute (2-h) ischemia (aISCH) and chronic (16-h) hypoglycemia (cHG). The antioxidants vitamin E and beta-carotene protected embryonic hippocampal cultures against 0-1600 mg/dl EtOH/aISCH/cHG treatments. In addition, neuronal viability, as measured by MTT ((3,4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; 5 mg/ml)), was equal to untreated cultures when supplemented with vitamin E or beta-carotene at 0-800 mg/dl or 0-200 mg/dl EtOH/aISCH/cHG, respectively. These in vitro studies mirror potential in utero ethanol-exposed CNS

conditions and may lead to therapeutic strategies targeted at attenuating neurodevelopmental FAS-related deficits.

Mitchell JJ, Paiva M, Moore DB, Walker DW, Heaton MB. **A comparative study of ethanol, hypoglycemia, hypoxia and neurotrophic factor interactions with fetal rat hippocampal neurons: a multi-factor in vitro model developmental ethanol effects.** Brain Res Dev Brain Res 1998 Feb 10;105 (2):241-50.
Fetal alcohol syndrome (FAS) is characterized by numerous central nervous system anomalies, with the hippocampus being particularly vulnerable to developmental ethanol exposure. In addition to direct ethanol neurotoxicity, other conditions resulting from maternal ethanol consumption, such as hypoglycemia and hypoxia, may also contribute to FAS. The present study used a tissue culture system to model multiple conditions which may relate to in vivo FAS, and assessed their relative neurotoxicity with MTT assays. Gestational day 18 rat hippocampal cultures were exposed to varying ethanol concentrations, glucose withdrawal-induced hypoglycemic (gwHG, 16 h) or acute hypoxic (aHP, 2 h) conditions alone, as well as to co-treatments with ethanol and gwHG or aHP. Brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) have previously been shown to ameliorate ethanol-, hypoglycemia- and hypoxia-induced neurotoxicity. Therefore, their neuroprotective potential, along with ciliary neurotrophic factor (CNTF), was examined. Neuronal viability was reduced dose-dependently by ethanol, alone or with hypoglycemia or hypoxia. Ethanol + gwHG or aHP was not uniformly additive. NGF treatment provided the most extensive neuroprotection, being effective against ethanol (200 and 400 mg/dl), gwHG, and aHP, alone and combined. BDNF afforded similar protection, but not against ethanol + gwHG. CNTF protected only against aHP. CNTF + BDNF, previously shown to act synergistically, protected against ethanol + aHP up to 800 mg/dl ethanol, but not, paradoxically, against ethanol alone, gwHG, or ethanol + gwHG, all conditions BDNF alone protected against. This study demonstrated that several neurotrophic factors are capable of mitigating neurotoxicity associated with ethanol, hypoglycemia and hypoxia.

Monteverdi GH, Di Giulio R. **An enzyme-linked immunosorbent assay for estrogenicity using primary hepatocyte cultures from the channel catfish (Ictalurus punctatus).** Arch Environ Contam Toxicol 1999;37(1):62-9.
BIOSIS COPYRIGHT: BIOL ABS. An in vitro assay has been developed to screen for estrogenic activity of single chemicals or complex mixtures. This method combines primary hepatocyte cultures from the channel catfish (Ictalurus punctatus) with an enzyme-linked immunosorbant assay (ELISA) to detect and quantify the production of vitellogenin (VTG), a liver-derived, estrogen-induced lipoprotein. A variety of environmentally relevant chemicals and chemical mixtures were tested, including the polyaromatic hydrocarbon benzo(a)pyre xifen) and 7alpha-(9-(4,4,5,5,5-pentafluoro-pentyl-sulfinyl) nonyl)estra-3,17- beta-diol (ICI-182,780) were also examined. The following compounds were observed to be estrogenic: DES > E2igmastanol, tamoxifen, ICI-182,780, and four paper mill effluents exhibited no detectable estrogenic activity. Furthermore, both tamoxifen and ICI-182,780 significantly reduced VTG synthesis by cells incubated with E2 or DES. Stigmastanol and the mill effluents were also tes those reported for VTG assays for other teleost species. Thus, the present work provides a sensitive, rapid means for screening the estrogenic potency of environmentally relevant chemicals and chemical mixtures in vitro.

Nagel SC, Vom Saal FS, Welshons WV. **Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum.** J Steroid Biochem Mol Biol 1999;69(1-6):343-57.
Many estrogenic chemicals found in the environment (xenoestrogens) show a lower affinity for plasma estrogen binding proteins relative to the natural estrogens such as estradiol. These binding proteins, which include alphafetoprotein in rats and mice, sex hormone binding globulin in humans, and albumin in all species, regulate estrogen uptake into tissues. Therefore, the in vivo estrogenic potency relative to estradiol of xenoestrogens that show lower binding to these serum proteins will thus be underestimated in assays that compare the potency of xenoestrogens to estradiol and do not take serum binding into account. We have examined the effects of the binding components in serum on the uptake of a number of xenoestrogens into intact MCF-7 human breast cancer cells. Since most estrogenic chemicals are not available in radiolabeled form, their uptake is determined by competition with [3H]estradiol for binding to estrogen receptors (ER) in an 18-h assay. Serum modified access (SMA) of cell uptake of xenoestrogens is calculated as the RBA in serum-free-medium divided by the RBA in serum, and the bioactive free fraction of xenoestrogen in serum is then also calculated. We predicted the concentration of two xenoestrogens, bisphenol A and octylphenol, required to alter development of the prostate in male mouse fetuses. Whereas octylphenol was predicted to be a more potent estrogen than bisphenol A when tested in serum-free medium, our assay predicted that bisphenol A would be over 500-times more potent than octylphenol in fetal mice. The finding that administration of bisphenol A at a physiologically relevant dose predicted from our in vitro assay to pregnant mice from gestation day 11 to 17 increased adult prostate weight in male offspring relative to controls (similar to the effect of estradiol), while the same doses of octylphenol did not alter prostate development, provided support for our hypothesis.

O'Brien ML, Park K, In Y, Park-Sarge OK. **Characterization of estrogen receptor-beta (ERbeta) messenger ribonucleic acid and protein expression in rat granulosa cells.** Endocrinology 1999;140 (10):4530-41.
We have examined estrogen-responsiveness of ovarian granulosa cells by focusing on estrogen receptor (ER) expression. Estrogen responsiveness was determined by examining the effect of 17beta-estradiol (1-10 nM) on luciferase reporter activity in rat granulosa cells transfected with an ERE-luciferase construct. The results demonstrate an estrogen-induced (approximately 3-fold) increase in luciferase reporter activity, indicating that granulosa cells contain functional estrogen response element (ERE)-binding transcriptional activators. Gel mobility shift assays in combination with ER antibodies show that ERbeta is the predominant ERE-binding protein in granulosa cells. Western blotting results show that granulosa cells contain ERbeta-immunoreactive protein(s) migrating at a size substantially larger than the recombinant protein generated from the originally proposed 485 amino acid open-reading frame. This size discrepancy is not due to granulosa cell expression of ERbeta isoforms with insertions within the coding region because RT-PCR assays revealed products with sizes expected for ERbeta, ERbetaB, and delta3 isoforms. This size discrepancy appears to be due to usage of a well-conserved, upstream in-frame translation initiation codon (ATG436) leading to a 530 amino acid open reading frame. ERbeta messenger RNA (mRNA) characterization using 5'-rapid amplification of complementary DNA ends (5'-RACE) show the presence of two different (P1- and P2-) 5'-ends of rat ERbeta mRNA encoding the full-length ERbeta protein. The generation of the P2-specific exon is likely due to initiation of transcription

from an alternative promoter. Both P1- and P2-specific exon-containing ERbeta mRNAs are expressed in granulosa cells, and they are rapidly down-regulated by the cAMP-mediated intracellular signaling pathway in cultured granulosa cells. Taken together, our results show that rat granulosa cells produce two different 3',5'-cAMP-regulated ERbeta mRNA species and that these mRNA species are capable of encoding the full-length ERbeta protein.

O'Connor JC, Frame SR, Biegel LB, Cook JC, Davis LG. **Sensitivity of a Tier I screening battery compared to an in utero exposure for detecting the estrogen receptor agonist 17 beta-estradiol.** Toxicol Sci 1998 Aug;44(2):169-84.
A Tier I screening battery for detecting endocrine active compounds (EACs) has been evaluated for its ability to identify 17 beta-estradiol, a pure estrogen receptor agonist. In addition, the responses obtained with the Tier I battery were compared to the responses obtained from F1 generation rats from a 90-day/one-generation reproduction study with 17 beta-estradiol in order to characterize the sensitivity of the Tier I battery against the sensitivity of an in utero exposure for detecting EACs. The Tier I battery incorporates two short-term in vivo tests (5-day ovariectomized female battery; 15-day intact male battery) and an in vitro yeast transactivation system (YTS) for identifying compounds that alter endocrine homeostasis. The Tier I female battery consists of traditional uterotrophic endpoints coupled with biochemical and hormonal endpoints. It is designed to identify compounds that are estrogenic/antiestrogenic or modulate dopamine levels. The Tier I male battery consists of organ weights coupled with microscopic evaluations and a comprehensive hormonal assessment. It is designed to identify compounds that have the potential to act as agonists or antagonists to the estrogen, androgen, progesterone, or dopamine receptor; steroid biosynthesis inhibitors (aromatase, 5 alpha-reductase, and testosterone biosynthesis); or compounds that alter thyroid function. The YTS is designed to identify compounds that bind to steroid hormone receptors (estrogen, androgen, and progesterone) and activate gene transcription. The profile generated for 17 beta-estradiol was characteristic of the responses expected with a pure estrogen receptor agonist. In the female battery, responses to 17 beta-estradiol included increases in uterine fluid imbibition, uterine weight, estrus conversion, uterine stromal cell proliferation, uterine epithelial cell height, uterine progesterone receptor content, serum prolactin and estradiol levels, and decreases in uterine estrogen receptor content and follicle stimulating hormone and luteinizing hormone levels. In the male battery, responses to 17 beta-estradiol included decreases in absolute testis and epididymides weights, decreases in relative weights for androgen-dependent tissues (prostate, seminal vesicles, and accessory sex gland unit), hormonal alterations (decreased serum testosterone, dihydrotestosterone, and LH and increased serum prolactin levels), and microscopic alterations of the testis and epididymides. In the YTS for the estrogen receptor, 17 beta-estradiol had an EC50 value of 7.2 x 10(-9) M, while DHT and progesterone had little cross-activation. The androgen and progesterone receptor systems were less selective in that 17 beta-estradiol activated these systems within 3 orders of magnitude of the primary ligand. In the 90-day/one-generation reproduction study, responses to dietary administration of 17 beta-estradiol included alterations in organ weights, developmental landmarks, and hormonal levels. Comparison of the responses obtained with our Tier I battery and an in utero exposure demonstrates that the Tier I screening battery is as sensitive as an in utero exposure for detecting 17 beta-estradiol-induced alterations in hormonal homeostasis.

O'Connor JC, Frame SR, Davis LG, Cook JC. **Detection of the environmental antiandrogen p,p-DDE**

in CD and Long-Evans rats using a tier I screening battery and a Hershberger assay. Toxicol Sci 1999;51(1):44-53.
In this report, p,p'-DDE, a weak androgen receptor (AR) antagonist, has been examined in a Tier I screening battery designed to detect endocrine-active compounds (EACs). The screening battery that was used to examine p,p'-DDE was an abbreviated version of a proposed Tier I screening battery (Cook et al., 1997, Regul. ToxicoL Pharmacol. 26, 60-68) that consisted of a 15-day intact male in vivo battery and an in vitro yeast transactivation system (YTS). In addition, strain sensitivity differences were evaluated using male Crl:CDIGS BR (CD) and Long-Evans (LE) rats. Finally, p,p'-DDE was examined in a Hershberger assay designed to detect AR agonists. In the in vivo male battery using CD rats, responses to p,p'-DDE included organ weight changes (increased relative liver weight and decreased absolute epididymis weight) and hormonal alterations (increased serum estradiol [E2] levels and decreased serum FSH and T4 levels). Responses to p,p'-DDE in LE rats included organ weight changes (increased relative liver weight, absolute epididymis weight, relative accessory sex gland [ASG] unit weight, as well as the individual component weights of the ASG [prostate and seminal vesicles]), and hormonal alterations (increased serum testosterone [T], E2, dihydrotestosterone [DHT], thyroid-stimulating hormone [TSH], and decreased T4 levels). These data demonstrate that there are considerable strain-sensitivity differences to p,p'-DDE exposure. The described in vivo male battery using CD rats did not identify p,p'-DDE as an EAC. In contrast, the in vivo male battery using LE rats identified p,p'-DDE as a EAC. Evaluation of the data for the LE rats demonstrate that p,p'-DDE appears to be acting as an AR antagonist whose primary effects are more potent centrally than peripherally. In the YTS for the AR, p,p'-DDE had an EC50 value of 3.5 x 10(-4) M; however, in the AR YTS competition assay, p,p'-DDE did not inhibit DHT binding to the AR. p,p'-DDE was inactive in the YTS containing the estrogen receptor or progesterone receptor at the concentrations evaluated. In the Hershberger assay, p,p'-DDE administration caused antiandrogen-like effects characterized by attenuation of the testosterone propionate-induced increases in reproductive-organ weights. In summary, these data suggest that strain selection will affect the ability to detect certain weak EACs. However, a Tier I screening battery consisting of both in vivo and in vitro endpoints would reduce the chance that weak-acting compounds such as p,p'-DDE would not be identified as potential EACs.

O'Connor JC, Frame SR, Davis LG, Cook JC. **Detection of thyroid toxicants in a tier I screening battery and alterations in thyroid endpoints over 28 days of exposure.** Toxicol Sci 1999;51(1):54-70.
Phenobarbital (PB), a thyroid hormone excretion enhancer, and propylthiouracil (PTU), a thyroid hormone-synthesis inhibitor, have been examined in a Tier I screening battery for detecting endocrine-active compounds (EACs). The Tier I battery incorporates two short-term in vivo tests (5-day ovariectomized female battery and 15-day intact male battery using Sprague-Dawley rats) and an in vitro yeast transactivation system (YTS). In addition to the Tier I battery, thyroid endpoints (serum hormone concentrations, liver and thyroid weights, thyroid histology, and UDP-glucuronyltransferase [UDP-GT] and 5'-deiodinase activities) have been evaluated in a 15-day dietary restriction experiment. The purpose was to assess possible confounding of results due to treatment-related decreases in body weight. Finally, several thyroid-related endpoints (serum hormone concentrations, hepatic UDP-GT activity, thyroid weights, thyroid follicular cell proliferation, and histopathology of the thyroid gland) have been evaluated for their utility in detecting thyroid-modulating effects after 1, 2, or 4 weeks of

treatment with PB or PTU. In the female battery, changes in thyroid endpoints following PB administration, were limited to decreased serum tri-iodothyronine (T3) and thyroxine (T4) concentrations. There were no changes in thyroid stimulating hormone (TSH) concentrations or in thyroid gland histology. In the male battery, PB administration increased serum TSH and decreased T3 and T4 concentrations. The most sensitive indicator of PB-induced thyroid effects in the male battery was thyroid histology (pale staining and/or depleted colloid). In the female battery, PTU administration produced increases in TSH concentrations, decreases in T3 and T4 concentrations, and microscopic changes (hypertrophy/hyperplasia, colloid depletion) in the thyroid gland. In the male battery, PTU administration caused thyroid gland hypertrophy/hyperplasia and colloid depletion, and the expected thyroid hormonal alterations (increased TSH, and decreased serum T3 and T4 concentrations). The dietary restriction study demonstrated that possible confounding of the data can occur with the thyroid endpoints when body weight decrements are 15% or greater. In the thyroid time course experiment, PB produced increased UDP-GT activity (at all time points), increased serum TSH (4-week time point), decreased serum T3 (1-and 2-week time points) and T4 (all time points), increased relative thyroid weight (2- and 4-week time points), and increased thyroid follicular cell proliferation (1- and 2-week time points). Histological effects in PB-treated rats were limited to mild colloid depletion at the 2- and 4-week time points. At all three time points, PTU increased relative thyroid weight, increased serum TSH, decreased serum T3 and T4, increased thyroid follicular cell proliferation, and produced thyroid gland hyperplasia/hypertrophy. Thyroid gland histopathology, coupled with decreased serum T4 concentrations, has been proposed as the most useful criteria for identifying thyroid toxicants. These data suggest that thyroid gland weight, coupled with thyroid hormone analyses and thyroid histology, are the most reliable endpoints for identifying thyroid gland toxicants in a short-duration screening battery. The data further suggest that 2 weeks is the optimal time point for identifying thyroid toxicants based on the 9 endpoints examined. Hence, the 2-week male battery currently being validated as part of this report should be an effective screen for detecting both potent and weak thyroid toxicants.

Osuga Y, Tsutsumi O, Momoeda M, Okagaki R, Matsumi H, Hiroi H, Suenaga A, Yano T, Taketani Y. **Evidence for the presence of hepatocyte growth factor expression in human ovarian follicles.** Mol Hum Reprod 1999;5(8):703-7.

The presence of hepatocyte growth factor (HGF) in follicular fluid (FF) relative to concentrations of sex steroid hormones and human chorionic gonadotrophin (HCG) was investigated. A total of 69 FF samples were obtained during oocyte retrieval for in-vitro fertilization (IVF) from 11 patients with no apparent endocrine disorders. The concentrations of HGF, oestradiol, progesterone, HCG and testosterone in FF samples were measured by enzyme-linked immunosorbent assay. Transcription of HGF and its receptor, c-met, was detected by reverse transcription-polymerase chain reaction (RT-PCR). Human FF samples contained approximately 90-fold higher amounts of HGF (24.2 +/- 1.2 ng/ml), compared with those of serum (0. 28 +/- 0.04 ng/ml). Concentrations of HGF in FF were positively correlated with those of progesterone ($r = 0.649$, $P < 0.0001$) and HCG ($r = 0.264$, $P = 0.026$) concentrations in FF. However, HGF concentrations were not significantly correlated with oestradiol and testosterone. HGF in FF was detected by Western blotting, as a single 90 kDa band, corresponding to a single chain form. Additionally, mRNA for both HGF and its receptor were detected in a crude granulosa cell preparation from the pre-ovulatory follicles. These findings suggest that HGF is produced locally in human ovarian follicles and may have a physiological role as an autocrine/paracrine factor.

Paksy K, Forgacs Z, Gati I. **In vitro comparative effect of Cd2+, Ni2+, and Co2+ on mouse postblastocyst development.** Environ Res 1999;80(4):340-7.
Postblastocyst development of mouse preembryos was studied in vitro in order to determine direct effect of Cd2+, Ni2+, and Co2+ ions on embryogenesis during the peri-implantation stage. Uterine horns were flushed on Day 4 of pregnancy and expanded blastocysts were cultured for 4 days in the presence of micromolar Cd2+ (1.1-26.4), Ni2+ (0. 1-500) or Co2+ (1-200). Area of trophoblast outgrowth was measured and used as a quantitative toxicological endpoint. Hatching, attachment, outgrowth, and formation of inner cells mass were also registered. Significant adverse effect on the development stages were observed at 2.2 microM (Cd2+), at 10 microM (Ni2+), and at 100 microM (Co2+). Cd2+ and Co2+ decreased the area of trophoblast markedly at concentrations of 1.1 and 10 microM, respectively. Ni2+ exposure resulted in a slight increase at 10 microM followed by a marked reduction in the trophoblast area at 250 microM. Reduced proliferative ability of trophoblast cells may point to compromised invasiveness of the embryo. The lowest Cd2+ concentration (1.1 microM=0.25 microg/ml) significantly deteriorating trophoblast development was found to be lower than Cd levels ranging up to 0.512 microg/g, reported in clinical ovarian samples of occupationally nonexposed women. The morphological alteration and loss of cellular contacts in blastocysts induced by exposure to Cd2+, Ni2+, or Co2+ may adversely influence adhesion and recognition events and may disturb aggregation of mononuclear trophoblastic cells to multinucleated cells in the course of peri-implantation in vivo as well. Copyright 1999 Academic Press.

Patyna PJ, Davi RA, Parkerton TF, Brown RP, Cooper KR. **A proposed multigeneration protocol for Japanese medaka (Oryzias latipes) to evaluate effects of endocrine disruptors.** Sci Total Environ 1999;233(1-3):211-20.
Definitive data on reproductive impairment of chronically exposed populations may be required to assess the appropriateness of the existing test methods for hazard identification and prioritization of endocrine modulators. Multigeneration toxicity testing protocols for wildlife receptors are lacking. To help address this gap we describe a multigeneration fish assay using the freshwater fish, Japanese medake (Oryzias latipes). This test species has been used for the evaluation of carcinogenic, teratogenic and reproductive effects and is sensitive to estrogen exposure producing ovo-testis, altered biochemical parameters and phenotypic characteristics. Due to the short life cycle, a multigeneration test with medaka can be conducted in 1 year. Endpoints evaluated include: survival, growth, sex ratio, fecundity, embryonic lesion occurrence, embryonic stage development, gonadal and hepatic somatic indices, histopathology and biochemical parameters. As new endpoints are developed they can be incorporated into the protocol. Results of a positive control (17 beta-estradiol) study are presented to give an indication of the baseline associated with various test endpoints and to highlight the importance of nutrition in the experimental design. 17 beta-Estradiol treatment induced vitellogenin production in male and female medaka, feminized males, and disrupted egg production. The proposed protocol provides researchers with an effective multigeneration fish test that can be used to examine potential effects of stressors at the population, individual, cellular and subcellular level.

Pickford DB, Morris ID. **Effects of endocrine-disrupting contaminants on amphibian oogenesis: methoxychlor inhibits progesterone-induced maturation of Xenopus laevis oocytes in vitro.** Environ Health Perspect 1999;107(4):285-92.

There is currently little evidence of pollution-induced endocrine dysfunction in amphibia, in spite of widespread concern over global declines in this ecologically diverse group. Data regarding the potential effects of endocrine-disrupting contaminants (EDCs) on reproductive function in amphibia are particularly lacking. We hypothesized that estrogenic EDCs may disrupt progesterone-induced oocyte maturation in the adult amphibian ovary, and tested this with an in vitro germinal vesicle breakdown assay using defolliculated oocytes from the African clawed frog, Xenopus laevis. While a variety of natural and synthetic estrogens and xenoestrogens were inactive in this system, the proestrogenic pesticide methoxychlor was a surprisingly potent inhibitor of progesterone-induced oocyte maturation (median inhibitive concentration, 72 nM). This inhibitory activity was specific to methoxychlor, rather than to its estrogenic contaminants or metabolites, and was not antagonized by the estrogen receptor antagonist ICI 182,780, suggesting that this activity is not estrogenic per se. The inhibitory activity of methoxychlor was dose dependent, reversible, and early acting. However, washout was unable to reverse the effect of short methoxychlor exposure, and methoxychlor did not competitively displace [3H] progesterone from a specific binding site in the oocyte plasma membrane. Therefore, methoxychlor may exert its action not directly at the site of progesterone action, but downstream on early events in maturational signaling, although the precise mechanism of action is unclear. The activity of methoxychlor in this system indicates that xenobiotics may exert endocrine-disrupting effects through interference with progestin-regulated processes and through mechanisms other than receptor antagonism.

Pineau C, Dupaix A, Jegou B. **The co-culture of Sertoli cells and germ cells: applications in toxicology.** Toxicol In Vitro 1999;13(4-5):513-20.
BIOSIS COPYRIGHT: BIOL ABS. Spermatogenesis is a very complex process by which male germ cells differentiate into mature spermatozoa. In this regard, the local regulation of spermatogenesis can be considered as a particular cellular achievement. This sophisticated communication network has its weak points, such that the dysfunctionment of one cell type propagates to all other cell types as a cascade. This explains the particular vulnerability of the testis to environmental factors, and more specifically drugs and xenobioti been the ones most usually used for the in vitro analysis of toxic compounds. While Sertoli cells are used in vitro for mechanistic toxicology studies, the extreme fragility of germ cells prevents their culture for that purpose. However, Sertoli and germ cells can be cultured together for short periods of time. This review presents the different in vitro testicular systems at disposal and provides examples of mechanistic studies undertaken to verify and deepen in vivo observations on the target.

Pineiro V, Casabiell X, Peino R, Lage M, Camina JP, Menendez C, Baltar J, Dieguez C, Casanueva F. **Dihydrotestosterone, stanozolol, androstenedione and dehydroepiandrosterone sulphate inhibit leptin secretion in female but not in male samples of omental adipose tissue in vitro: lack of effect of testosterone.** J Endocrinol 1999;160(3):425-32.
Leptin, the product of the Ob gene, is a polypeptide hormone expressed in adipocytes which acts as a signalling factor from the adipose tissue to the central nervous system, regulating food intake and energy expenditure. It has been reported that circulating leptin levels are higher in women than in men, even after correction for body fat. This gender-based difference may be conditioned by differences in the levels of androgenic hormones. To explore this possibility, a systematic in vitro study with organ cultures from human omental adipose tissue, either stimulated or not with androgens (1 microM), was

undertaken in samples obtained from surgery on 44 non-obese donors (21 women and 23 men). The assay was standardized in periods of 24 h, ending at 96 h, with no apparent tissue damage. Leptin results are expressed as the mean+/-s.e.m. of the integrated secretion into the medium, expressed as ng leptin/g tissue per 48 h. Spontaneous leptin secretion in samples from female donors (4149+/-301) was significantly higher (P<0.01) than that from male donors (2456+/-428). Testosterone did not exert any significant effect on in vitro leptin secretion in either gender (4856+/-366 in women, 3322+/-505 in men). Coincubation of adipose tissue with dihydrotestosterone (DHT) induced a significant (P<0.05) leptin decrease in samples taken from women (3119+/-322) but not in those taken from men (2042+/-430). Stanozolol, a non-aromatizable androgen, decreased (P<0.05) leptin secretion in female samples (2809+/-383) but not in male (1553+/-671). Dehydroepiandrosterone sulphate (DHEA-S) induced a significant (P<0.01) leptin decrease in female samples (2996+/-473), with no modifications in samples derived from males (1596+/-528). Exposure to androstenedione also resulted in a significant reduction (P<0.01) of leptin secretion in samples taken from women (2231+/-264), with no effect on male adipose tissue (1605+/-544). In conclusion, DHT, stanozolol, DHEA-S and androstenedione induced a significant inhibition of in vitro leptin secretion in samples from female donors, without affecting the secretion in samples from men. Testosterone was devoid of activity in either gender.

Piscopo SE, Smoak IW. **Comparison of effects of albendazole sulfoxide on in vitro produced bovine embryos and rat embryos.** Am J Vet Res 1997 Sep;58(9):1038-42.
OBJECTIVE: To evaluate and compare effects of albendazole sulfoxide (ABZSO) on rat embryos and bovine embryos produced in vitro. ANIMALS: In vitro produced bovine embryos. Rat embryos recovered from naturally bred Sprague-Dawley rats. PROCEDURE: 4- and 8-cell bovine embryos were randomly allocated to ABZSO or vehicle control groups. After 48 hours, embryos were evaluated for cell number and blastomere morphology. Rat embryos of similar stages, flushed from the uterine tube on gestational day 2-5, were randomly allocated to treatment or control groups. After 24 hours, embryos were evaluated as described previously. RESULTS: 44% of control bovine embryos divided in culture (> or = 16-cell stage). Fifteen percent of the controls had morphologic abnormalities, including disparity in blastomere size and cytoplasmic vacuoles and stippling. Treated (> or = 1 microgram of ABZSO/ml) bovine embryos differed (P < 0.0001) from controls, with 4% development and 93% abnormal morphology. Forty-five percent of control rat embryos divided in culture. Treated (> or = 500 ng of ABZSO/ml) rat embryos differed (P < 0.0003) from controls with regard to ability to divide. There were no consistent morphologic abnormalities in rat embryos. CONCLUSIONS: In vitro produced bovine embryos were susceptible to ABZSO at a concentration > or = 1 microgram/ ml, resulting in decreased ability to divide and presence of gross morphologic abnormalities. Rat embryos produced in vivo and exposed in vitro to ABZSO at a concentration > or = 500 ng/ml had decreased ability to divide in culture. CLINICAL RELEVANCE: Despite severe effects of ABZSO (> or = 1 microgram/ml) on bovine embryo development in vitro, it is beyond the scope of this study to speculate whether a therapeutic dosage of albendazole (10 mg/kg of body weight) would result in necessary concentrations of ABZSO in vivo to disrupt embryogenesis.

Pugarelli JE, Brent RL, Lloyd JB. **Effects of methionine supplement on methionine incorporation in rat embryos cultured in vitro.** Teratology 1999;60(1):6-9.
The effect of supplementary L-methionine (Met) on the incorporation of methionine was evaluated in

9.5-day rat conceptuses cultured in vitro. Parallel experiments with L-leucine (Leu) were performed for comparison. Conceptuses were cultured for 24 hr in the presence of 3H-labeled Met or Leu, and the incorporation of radiolabel into the embryo and visceral yolk sac was measured. Supplementary Met proportionately increased the incorporation of Met, but supplementary Leu did not have as great an effect on the incorporation of Leu. A hypothesis is presented to explain these findings. It is proposed that Met, but not Leu, is a rate-limiting nutrient for organogenesis-stage rat embryos cultured in rat serum. The results are also discussed with reference to the established efficacy of supplementary folic acid in decreasing the incidence of neural tube defects in human populations and to claims that Met reverses certain teratogenic phenomena, both in vitro and in vivo.

Rajadhyaksha AV, Reddy V, Hover CG, Kulkarni AP. **N-demethylation of phenothiazines by lipoxygenase from soybean and human term placenta in the presence of hydrogen peroxide.** Teratog Carcinog Mutagen 1999;19(3):211-22.
Several phenothiazine derivatives have been shown to cause reproductive toxicity. The biochemical mechanisms responsible for these effects are not fully understood at present. In this study, we investigated hydrogen peroxide-dependent oxidation of six phenothiazines by purified lipoxygenase from soybean (SLO) and human term placenta (HTPLO). Chlorpromazine was employed as the prototype phenothiazine drug. Chlorpromazine was easily demethylated releasing formaldehyde when incubated at pH 7.0 and 6.5 with SLO or HTPLO, respectively, in the presence of hydrogen peroxide. The reaction was linear with respect to time, exhibited dependence on the amount of enzyme, and the concentration of chlorpromazine and hydrogen peroxide. Under the optimal assay conditions, the estimated Vmax values for chlorpromazine N-demethylation were 139 and 7.2 nmoles/min/mg of SLO and HTPLO, respectively. Collectively, the results suggest an enzymatic nature of the reaction. In the presence of gossypol and NDGA, the classical inhibitors of different lipoxygenases, the formaldehyde production was significantly decreased, as expected. Similar to SLO, the generation of chlorpromazine cation radical, an initial oxidation product with an absorption maximum at 525 nm, was also observed with HTPLO. The radical generation was detectable only under acidic conditions (pH 3.5-4.5). The formaldehyde production was also decreased by BHT and BHA, suggesting a radical nature of the SLO-mediated chlorpromazine N-demethylation. Reduced glutathione, ascorbate, and dithiothreitol suppressed the rate of SLO-dependent formaldehyde generation, presumably due to the reduction of the cation radical back to chlorpromazine in a concentration-dependent manner. Besides chlorpromazine, SLO also oxidized promazine, triflupromazine, trifluperazine, trimeprazine, and perphenazine, albeit at different rates, in the presence of hydrogen peroxide. The evidence gathered in this in vitro study suggests that phenothiazines can undergo peroxidative N-demethylation via lipoxygenase pathway. The role of this biochemical mechanism in the in vivo developmental toxicity of phenothiazines remains to be established.

Rao SS, Metcalfe CD, Neheli TA, Schmidt B. **Assessing the toxicity of environmental contaminants with early life stages of Japanese medaka (Oryzias latipes).** Environ Toxicol Water Qual 1997 Nov;12(4):349-5.

Rieger D. **Effects of the in vitro chemical environment during early embryogenesis on subsequent development.** Arch Toxicol Suppl 1998;20:121-9.
The development of the preimplantation embryo seems morphologically very simple, and embryologists

previously assumed that an embryo that developed to the blastocyst stage was fully capable of normal development after transfer to the uterus of a recipient female. This complacency was disturbed by reports that exposure of early embryos to mutagens such as methylnitrosourea led to fetal abnormalities, decreased birth rates, and decreased life-span. Even more disturbing are recent reports that culture of early embryos in supposedly benign conditions can adversely affect their subsequent development. Techniques have been developed for the production of cattle and sheep embryos by in-vitro fertilization and by cloning. Such embryos must be cultured for several days before they can be transferred, and, in some cases, this has been related to abortion, very high birthweight, physical abnormalities and peri-natal mortality of the calves and lambs. This syndrome may result from an unbalanced development of the trophoblast relative to the inner-cell mass, possibly related to the presence of serum, glucose, or ammonium in the culture medium. An analogous phenomenon has been observed in human in-vitro fertilization where babies from single pregnancies have below-normal birth-weight. There is also evidence to suggest that the in-vitro environment of the gametes before fertilization can affect subsequent embryonal and fetal development. Exposure of mouse oocytes to vitrification solutions has been shown to lead to fetal malformations, and treatment of bull sperm with glutathione improves early embryo development. The common thread in these diverse observations is that development can be affected by events that occur long before any defect is apparent. Consequently, the production of a morphologically normal embryo is no guarantee that fetal development and post-natal life will be normal. This is of immediate concern in human reproductive medicine due to the increasing use of sperm injection for fertilization, and the emergence of in-vitro oocyte maturation. Further development and application of reproductive techniques would benefit from a toxicological evaluation of risk factors and exposure limits.

Robbins WA, Rubes J, Selevan SG, Perreault SD. **Air pollution and sperm aneuploidy in healthy young men.** Environ Epidemiol Toxicol 1999;1(2):125-31.
BIOSIS COPYRIGHT: BIOL ABS. Molecular genetic techniques including the sperm chromatin structure assay (SCSA), single cell gel assay (COMET), deoxynucleotidyl transferase-mediated nick end labeling assay (TUNEL), and fluorescence in situ hybridization (FISH) allow direct measurement of germ cell chromatin and cytogenetic damage in human sperm. A number of these technologies are proving to be useful when combined with epidemiological techniques in studies of environmentally induced germ cell damage. We report the use of one in the study were healthy, 18-year-old nonsmokers. Adjusting for potential confounders (alcohol, caffeine intake, fever, laboratory variables) did not change the effect estimate Incidence Rate Ratio (IRR) = 5.25, 95% CI = 2.5-11.0 (Poisson regression modeling). These findings suggest an effect of air pollution on human germ cell chromosomes that warrants further investigation.

Scholz G, Genschow E, Pohl I, Bremer S, Paparella M, Raabe H, Southee J, Spielmann H. **Prevalidation of the Embryonic Stem Cell Test (EST): a new in vitro embryotoxicity test.** Toxicol In Vitro 1999;13(4-5):675-81.
BIOSIS COPYRIGHT: BIOL ABS. Pluripotent embryonic stem cells (ES cells) of the mouse (cell-line D3) can be maintained in the undifferentiated state in the presence of LIF (Leukaemia Inhibitory Factor). Upon withdrawal of LIF, these cells differentiate into various cell types under appropriate conditions. This property of ES cells allowed us to develop an in vitro embryotoxicity test, the

Embryonic Stem Cell Test (EST; In Vitro Toxicology 1997, 10, 119-127), which does not require taking embryonic cells or tissues from preg n scheme. In the first stage of the study (Phase I), a standard operating procedure (SOP) was elaborated. In the second phase (Phase II), the interlaboratory transferability of the EST was assessed using three test chemicals representing three classes of embryotoxicity (a strong, a weak and a non-embryotoxic chemical) in two European laboratories (ZEBET at the BgVV in Berlin, Germany; ECVAM at the JRC in Ispra, Italy) and one US laboratory (Institute for In Vitro Sciences (IIVS) in Gaithersburgh.

Smeets JM, Rankouhi TR, Nichols KM, Komen H, Kaminski NE, Giesy JP, Van Den Berg M. **In vitro vitellogenin production by carp (Cyprinus carpio) hepatocytes as a screening method for determining (anti)estrogenic activity of xenobiotics.** Toxicol Appl Pharmacol 1999;157(1):68-76.
The yolk protein precursor vitellogenin (Vtg) is secreted by the liver of female as well as male fish, in response to estrogenic compounds. In this study, an in vitro assay was developed for measuring Vtg induction, using cultured primary hepatocytes from genetically uniform strains of carp (Cyprinus carpio). Vtg production was measured by indirect competitive ELISA, using a polyclonal antiserum against goldfish Vtg that cross-reacts with carp Vtg. Vtg was dose-dependently induced by 17beta-estradiol (E2) in hepatocytes of both sexes. E2 had a lowest observed effect concentration (LOEC) for Vtg induction of 2 nM, an EC50 between 50 and 150 nM, and a maximum response at 2 microM. The plasticizer and xenoestrogen bisphenol-A induced Vtg secretion by hepatocytes of both sexes at 50 and 100 microM. This carp hepatocyte (CARP-HEP) assay can also be used to detect antiestrogenic activity, which was measured as the reduction of E2-stimulated Vtg synthesis. Two well-known antiestrogenic compounds, tamoxifen and 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD), were tested. TCDD caused a reduction in Vtg synthesis in female hepatocytes at concentrations <0.1 nM, making it approximately 10,000-fold more potent than tamoxifen. Carp hepatocytes were also sensitive to induction of cytochrome P4501A (CYP1A) activity, measured as ethoxyresorufin O-deethylase (EROD). Depending on the exposure time, 18 or 96 h, EROD EC50 values for TCDD were 27 or 6 pM, respectively. The CARP-HEP assay, using the 96-well plate format, offers good possibilities to screen large numbers of compounds for (anti)estrogenic properties. In addition, it can simultaneously determine aryl hydrocarbon receptor agonist properties, measured as CYP1A induction. Copyright 1999 Academic Press.

Stevens JT, Gfeller W, Machemer L, Leist KH. **Adequacy of required regulatory hazard testing for the detection of potential hormonal activity of crop protection chemicals.** J Toxicol Environ Health B Crit Rev 1998 Jan-Mar;1(1):59-79.
The capacity of some synthetic chemicals, the so-called "endocrine-disrupting chemical," to alter hormonal activity, as well as the adequacy of the testing of chemicals to evaluate this capacity, has been called into question. Among the chemicals indicted have been certain crop protection agents or pesticides. Crop protection chemicals rank among the most closely regulated and thoroughly tested chemicals in use in both the human health and environmental hazard areas. However, it has been proposed that in vitro and in vivo screening tests be used to identify potential endocrine-active chemicals and to supplement or replace required regulatory bioassays. In vitro tests, such as receptor binding, examine a single chemical event, do not measure toxicity, post-receptor-mediated biological response, or the absorption, distribution, metabolism, and elimination of a chemical. Therefore, data derived solely from such a limited testing technique should not be used as a basis for selection of chemicals for making

regulatory decisions. In vivo screening tests, such as the uterotrophic assay, which promise to provide a rapid answer to a targeted question, do not capture the complexity of the biological response. As in the case with in vitro tests, results from a single in vivo test, such as a change in uterine weight, should not be used as a basis for regulatory decision making. Further, it has been suggested that such a screening battery should be put into place for ecotoxicity testing. Yet it is well recognized that endocrine-active chemicals that affect fish and wildlife in their natural habitat have been shown to cause similar adverse effects in laboratory test animals. Therefore, these screening tests do not add value to the current regulatory test battery. Evidence is presented that demonstrates that the regulatory safety assessment paradigm has a low likelihood of missing potential endocrine-active chemicals and has served society well.

Szczygiel M, Kurpisz M. **[Sperm morphology and xenogenic human sperm-hamster oocyte penetration assay in vitro]**. Ginekol Pol 1999;70(1):26-32. (Pol)
The aim of this study was to evaluate sperm morphology (teratozoospermia) and its effect on xenogenic human sperm-hamster oocyte penetration assay (HOPA). Sperm morphology assessment was performed in 140 infertile patients and 25 healthy individuals. According to Kruger's strict criteria teratozoospermia was diagnosed in 38 individuals out of 140 infertile males. HOPA assay was performed for 20 teratozoospermic patients and 10 healthy volunteers. No significant decrease in HOPA values was observed in cases of isolated teratozoospermia. The sperm anomaly severely influencing HOPA values was asthenozoospermia. We believe that evaluation of sperm morphology according to the strict criteria of Kruger should be applied for routine andrological practice which reflects rapidly changing 'normal' values of human semen.

Takahashi M, Saka N, Takahashi H, Kanai Y, Schultz RM, Okano A. **Assessment of DNA damage in individual hamster embryos by comet assay.** Mol Reprod Dev 1999;54(1):1-7.
DNA damage induced by either light exposure or oxidative stress likely contributes to the compromised development in vitro of cultured preimplantation embryos. Using the comet assay, which entails microgel electrophoresis that can readily detect single-strand breaks in DNA, a significant increase in DNA damage was detected in individual one-cell hamster embryos that were treated with either ultraviolet light or hydrogen peroxide. In addition, an increase in DNA damage also was observed following exposure of one-cell embryos to visible light. When the embryos were placed in drops of culture medium that were covered with mineral oil and the dishes then placed in a portable incubator containing 5% CO(2) in air at 37 degrees C, visible and UV light irradiation for 30 min still induced extensive DNA damage when compared to control embryos that were kept in the dark. In contrast, infrared irradiation did not induce an increase in DNA damage. DNA damage also was measured in individual one- and two-cell stage embryos developed in vivo or in vitro. The extent of DNA damage in the cultured embryos was significantly greater than in embryos that developed in vivo. These results highlight the usefulness of the comet assay to assess DNA damage in individual preimplantation embryos and how the assay can be used to monitor culture conditions in vitro. Copyright 1999 Wiley-Liss, Inc.

Tatone C, Francione A, Marinangeli F, Lottan M, Varrassi G, Colonna R. **An evaluation of propofol toxicity on mouse oocytes and preimplantation embryos.** Hum Reprod 1998 Feb;13(2):430-5.
Mouse biological assays were used to investigate potential adverse effects of propofol on the oocyte's

competence to fuse with spermatozoa and on the embryo's ability to develop to the blastocyst stage. Cumulus-enclosed metaphase II oocytes were exposed for 1 h to 0.01, 0.1, 0.4, 1 and 10 microg/ml propofol (Diprivan) and subjected to a sperm-oocyte fusion test based on the dye (Hoechst 33342) transfer technique. Oocytes exposed to 0.4, 1 and 10 microg/ml propofol showed a significant reduction in the rate of sperm fusion and underwent pronuclei formation at a rate similar to that of sperm fusion. In a second trial, mouse 1-cell and 2-cell embryos were exposed to varying propofol concentrations for 14h and then checked for subsequent development. Although adverse effects were not observed in 2-cell embryos, treatment of 1-cell embryos with propofol concentrations ranging from 0.01 to 10 microg/ml resulted in the inhibition of cleavage to blastocyst stage. We conclude that propofol can negatively influence fertilization in the mouse by impairing the oocyte's ability to fuse with spermatozoa, without interfering with the sperm-induced activation of the cell cycle. Moreover, we document the peculiar sensitivity to propofol of mouse 1-cell embryos as compared with 2-cell embryos.

Teramoto S, Takahashi KL, Kikuta M, Kobayashi H. **3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) as a direct-acting teratogen in micromass in vitro tests.** Congen Anomal 1999;39 (1):31-5.
BIOSIS COPYRIGHT: BIOL ABS. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) causes complete inhibition of rat embryonic midbrain (CNS) cell differentiation in the micromass in vitro test when applied at a concentration of 5 mug/ml under conditions where MX is rapidly degraded in culture medium with a half-life of 56 min. This study investigated whether or not degradation products of MX have inhibitory effects on CNS cell differentiation following pre-incubation of MX in culture medium for 0.5, 1 or 2 hr. When MX.

Tillner J, Nau H, Winckler T, Dingermann T. **Evaluation of the teratogenic potential of valproic acid analogues in transgenic Dictyostelium discoideum strains.** Toxicol In Vitro 1998 Aug;12(4):463-9.
Very early during the development of new pharmaceuticals toxicological tests are most important. In addition to acute and chronic toxicity tests, it is crucial to estimate the teratogenic potential of promising drugs. We established a simple biological test system based on the cellular slime mold Dictyostelium discoideum. Under certain environmental conditions single cells of D. discoideum aggregate and undergo a relatively simple cell differentiation program, leading to the formation of stalk and spore cells. Transgenic D. discoideum strains carrying the bacterial beta-galactosidase gene under the control of various developmentally regulated D. discoideum promoters were shown to be useful tools to test the teratogenic potential of valproic acid (VPA). This study describes the effects of the VPA analogues S-4-yn-VPA, R-4-yn-VPA, and 2-ethyl-4-pentynoic acid on the D. discoideum developmental system. The presence of S-4-yn-VPA during D. discoideum development resulted in a strong inhibition of spore cell differentiation, whereas stalk cell formation was less affected. The enantiomer R-4-yn-VPA as well as 2-ethyl-4-pentynoic acid had only moderate effects on D. discoideum development. The above results are consistent with data obtained in mammalian teratogenicity assays, and suggest that D. discoideum development should be investigated with a number of additional substances to provide a simple alternative for high throughput screenings of new drugs.

Titenko-Holland N, Shao J, Zhang L, Xi L, Ngo H, Shang N, Smith MT. **Studies on the genotoxicity of molybdenum salts in human cells in vitro and in mice in vivo.** Environ Mol Mutagen 1998;32(3):251-9.

Molybdenum is an essential element in plants and animals as a cofactor for enzymes. Molybdenum trioxide is used in metallurgical processes, in cosmetics as a pigment, and in contact lens solution, yet limited information is available on molybdenum genotoxicity. In the present study the micronucleus (MN) assay in human lymphocytes and mouse bone marrow and the dominant lethal assay in mice were used to assess the genotoxic effects of molybdenum salts in vitro and in vivo. Two salts of molybdenum were tested in whole blood cultures. Ammonium molybdate was more potent than sodium molybdate in causing a dose-dependent decrease in viability and replicative index and an increase in MN formation in binucleated lymphocytes ($P < 0.001$). A dose-response in both kinetochore-positive MN (caused by chromosome lagging) and kinetochore-negative MN (associated with chromosome breakage) was observed. Based on the results of a toxicity study of sodium molybdate, two doses, 200 and 400 mg/kg, were assessed in the bone marrow MN assay in mice (two i.p. injections 24 and 48 hr prior to euthanasia). A modest but statistically significant increase in MN frequency in polychromatic erythrocytes was observed ($P < 0.05$). The same treatment protocol was used to analyze dominant lethality. A dose-dependent increase in postimplantation loss represented mostly by early resorptions was observed the first week after treatment ($P = 0.003$). These preliminary data suggest that sodium molybdate induces dominant lethality at the postmeiotic stage of spermatogenesis. Overall, molybdenum salts produced moderately positive results both in vitro in human cells and in vivo in mice.

Ulbrich B, Palmer AK. **Neurobehavioral aspects of developmental toxicity testing.** Environ Health Perspect 1996 Apr;104 Suppl 2:407-12.
Tests for detection of neurobehavioral changes in the offspring have been a regulatory requirement in developmental toxicity testing of drugs for almost 20 years. Keeping their purpose of hazard identification and risk assessment for humans in mind, investigators and agency reviewers have become deeply ingrained with some stereotyped behaviors with respect to such relevant issues as choice of animal species and data evaluation. Other problematic areas of study design and conduct, selection of litter representatives for testing, what methods to combine in a testing battery, and statistical treatment of results and their interpretation, will need more research and discussion in the future.

Vakharia D, Gierthy J. **Rapid assay for oestrogen receptor binding to PCB metabolites.** Toxicol In Vitro 1999;13(2):275-82.
BIOSIS COPYRIGHT: BIOL ABS. Hydroxylated metabolites of polychlorinated biphenyls (PCBs) bind the oestrogen receptor (ER) and increase uterine weight in animal models. This oestrogenic action of PCB metabolites is of putative human health concern. We have thus developed a system to study human in vitro PCB metabolism. Human liver microsomes produced PCB metabolites in an NADPH-dependent microsomal reaction mixture (MRM); the metabolites were tested for ER-binding affinity in the human recombinant oestrogen receptor-alpha (ent of the 246BP-MRM had a retention time identical to that of 4'OH-246BP using HPLC. This method provides a rapid screen for the qualitative assessment of the presence of microsomally-generated ER-binding PCB metabolites to guide further characterization of these compounds by GC/MS analysis.

Van Maele-Fabry G, Gofflot F, Picard JJ. **Defects in the development of branchial nerves and ganglia induced by in vitro exposure of mouse embryos to mercuric chloride.** Teratology 1996 Jan;53(1):10-20.
The embryotoxic and dysmorphogenic effects of mercuric chloride ($HgCl_2$) have been studied in mouse

embryos cultured in vitro. In addition, the alterations induced in the developing branchial nerves and ganglia were analyzed. Mouse embryos with 6-8 pairs of somites were exposed for 26 hr to increasing concentrations (0, 12.5, 25, 50 microM) of HgCl2. After this period, a first set of embryos was removed and a second set of embryos transferred to culture medium without HgCl2 and remained in culture for an additional 22 hr. Both sets of embryos were examined for (1) survival, (2) presence of external dysmorphogenesis, (3) growth, and (4) differentiation. Dose-related alterations of these parameters were observed. The main target was the cephalic neural tube (mainly the forebrain), but several other systems were also affected (e.g., the turning of the embryos, the optic system). The 48-hr cultured embryos were immunostained using a monoclonal antineurofilament antibody to visualize defects in the development of branchial nerves and ganglia. HgCl2 induced a pronounced retardation in the differentiation of ganglion/nerve V and a slight retardation in the differentiation of ganglia/nerves VII and IX. The ganglia/nerves VIII and X were not retarded. In addition, hight percentages of abnormalities of ganglion/nerve V and fusions between ganglia/nerves IX and X were observed in these embryos. Disorganized fibers between ganglia/nerves VII-VIII and IX and between ganglia/nerves IX and X were also more frequently observed. At the highest concentration, asymmetric defects were induced by HgCl2 with a more pronounced effect observed on the right side of the embryos. These results demonstrate the usefulness of this approach in evaluating the susceptibility of the developing branchial nerves to the adverse effects of developmental toxicants.

Vesely D, Vesela D, Jelinek R. **The bioavailability of substances administered to chick embryos: the maximum effective route of administration.** Alternatives Lab Anim 1997 Nov/Dec;25(6):655-65. Toxicokinetic studies are of key importance in both the design and the interpretation of developmental toxicity studies. The aim of this study was to determine concentrations of test substances within the chick embryo following the administration schedule recommended in the chick embryotoxicity screening test (CHEST). The concentration-time relationships were investigated by using four labelled substances with various physicochemical and embryotoxic properties ((14C) sodium acetate, (14C) palmitic acid, (3H) cortisol and (3H) cytosine arabinoside). These labelled chemicals were mixed with cold substances and singly administered at two dose levels to chick embryos on days 2, 3 and 4 of incubation. Extrachorial and subgerminal routes were used on day 2, and extrachorial and intra-amniotic applications were chosen on days 3 and 4. The concentration of labelled chemical present within the embryo was assessed at predetermined intervals by scintillation fluorimetry (from 6 minutes to 96 hours after administration), and used for estimating the concentration curves. Regardless of the substance, dose and application route, the concentration curves exhibited a characteristic pattern, reaching their peaks within the first 6 hours, and dropping down to near zero 48-96 hours after administration. The decrease followed the first order law, demonstrating that, within the CHEST system, the avian embryo does not act as a closed system. With regard to the total amount of substance entering the embryo, extrachorial administration appeared to be superior to subgerminal administration on day 2. Intra-amniotic administration was superior to extrachorial administration on days 3 and 4. These differences were most pronounced after administration of lipid-soluble palmitic acid. The concentrations within embryonic tissues were directly dose-dependent. After consideration of all these findings, we concluded that the CHEST system probably has closer similarity to the toxicokinetics of exposure of mammalian embryos (i.e. reaching a peak and then a gradual decline over time) than any other in vitro test of developmental toxicity, where the chemical is simply added to culture media. Several practical recommendations for

improving the CHEST system were derived.

Webster WS, Brown-Woodman PD, Ritchie HE. **A review of the contribution of whole embryo culture to the determination of hazard and risk in teratogenicity testing.** Int J Dev Biol 1997 Apr;41 (2):329-35.
Whole embryo culture appears to be an excellent method to screen chemicals for teratogenic hazard. Compared to in vivo testing it is cheap and rapid and does not involve experimentation on live adult animals. Also in the important area of risk estimation whole embryo culture offers distinct advantages over in vivo teratogenicity testing. Adverse embryonic outcomes (malformations or embryotoxicity) are directly related to the serum concentration of the compound being tested and can be compared to the serum concentration in the human. A similar comparison is not possible after in vivo testing because for most compounds there are major pharmacokinetic differences between humans and experimental animals. In vivo testing is also limited by the possibility that metabolites that occur in the human do not occur in the test animal. This problem can be overcome in the in vitro system by adding the metabolite directly at the desired concentration either with or without the parent compound. There is only one major disadvantage to in vitro testing and that is the limited period of embryogenesis that is undertaken in the commonly used culture system. This restricts the range of malformations that can be induced and may render the testing system unsuitable for compounds that are likely to exert their major toxicological effect late in gestation. Any evaluation of whole embryo culture for hazard and risk assessment in teratology must take into account the limited value of currently used in vivo methods. Over 2000 chemicals have been reported to be teratogenic in experimental animals exposed in vivo (Shepard, Catalog of Teratogenic Agents, 1989). In comparison only about 20 chemicals are known to cause birth defects in the human. This large number of in vivo false-positive cannot easily be distinguished from true-positives. In this respect in vivo testing is severely deficient. The embryo culture testing system would also be expected to produce many false-positives; but by comparing effective drug concentrations with human therapeutic concentrations they can be differentiated from true-positives. The most serious deficiency for an in vivo or in vitro teratogenicity testing system would be false-negatives. This has not been a problem in the validation of in vitro testing so far (except perhaps procarbazine), but difficult drugs such as thalidomide were not included. Thalidomide remains an important index chemical because it is not teratogenic in rats or mice but is teratogenic in the rabbit and human. It is likely that these species differences are due to metabolic differences between species and it is possible that if the proximate teratogen/s of thalidomide were identified they would be teratogenic in rat embryo culture. Whole embryo culture remains a very powerful technique that should continue to contribute to the determination of the safety of drugs and other chemicals during pregnancy.

Whittington K, Ford WC. **Relative contribution of leukocytes and of spermatozoa to reactive oxygen species production in human sperm suspensions.** Int J Androl 1999;22(4):229-35.
The contribution of leukocytes and of spermatozoa to reactive oxygen species (ROS) production in prepared sperm suspensions from donors and subfertility patients was compared. In both groups, more leukocytes/10(6) spermatozoa were counted in samples which produced detectable ROS than in those that did not: Donors-645 vs. 170 (medians, $n = 7$; $p < 0.01$, Kruskal-Wallis), Subfertile group-1785 ($n = 18$) vs. 11 ($n = 8$) ($p < 0.005$, Kruskal-Wallis), respectively. Leukocyte concentrations were correlated with basal ($r = 0.826$, $p < 0.001$) and with ROS production stimulated with 50 mumol N-formyl, met,

leu, phe l-1 (N-FMLP) ($r = 0.835$, $p < 0.001$) and 100 nmol phorbol 12-myristate 13-acetate l-1 (PMA) ($r = 0.835$, $p < 0.001$) measured using a chemiluminescence assay. Leukocytes were removed from the sperm suspensions of 6 donors and from 96 ejaculates from 21 subfertility patients and ROS production was determined. Subsequently, in all 6 donors, N-FMLP did not stimulate ROS production indicating that leukocyte removal was complete, though in one case PMA stimulated low levels of ROS production. In 65 ejaculates from subfertile men the N-FMLP response was completely eliminated but in 7 of these samples PMA continued to stimulate ROS production. We conclude that infiltrating leukocytes are the predominant source of ROS production in unpurified sperm preparations. Some purified sperm suspensions could be stimulated to produce ROS by the addition of PMA indicating that spermatozoa themselves may produce ROS, albeit in much smaller amounts.

Yang ZM, Paria BC, Dey SK. **Activation of brain-type cannabinoid receptors interferes with preimplantation mouse embryo development.** Biol Reprod 1996 Oct;55(4):756-61.
The recent identification and cloning of guanine nucleotide regulatory protein-coupled brain-type and spleen-type cannabinoid receptors (CB1-R and CB2-R, respectively) provide evidence that many of the effects of cannabinoids are mediated via these receptors. Our recent observation of expression of both CB1-R and CB2-R genes in the preimplantation mouse embryo suggests that it could also be a target for cannabinoids. Indeed, cannabinoid agonists interfered with preimplantation embryo development in vitro. To examine whether cannabinoid effects on preimplantation embryos are mediated via CB1-R, we developed rabbit antipeptide antibodies against the N-terminal region of CB1-R and examined the receptor protein in the blastocyst by Western blotting and its spatiotemporal distribution in preimplantation mouse embryos by immunohistochemistry. Cannabinoid binding sites in the blastocyst were examined by Scatchard analysis, while the reversibility of cannabinoid-induced embryonic arrest in vitro was monitored using a specific antagonist to CB1-R, SR141716A. Western blot analysis detected a major band of approximately 59 kDa and a minor band of approximately 54 kDa in the blastocyst. Immunocytochemistry detected this receptor protein from the 2-cell through the blastocyst stages. Scatchard analysis using 3H-anandamide (an endogenous ligand) showed a single class of binding sites in Day 4 blastocysts with an apparent Kd of 1.0 nM and Bmax of 0.09 fmol/blastocyst. Considering the total number of cells (approximately 50) and total protein content (approximately 20 ng) of a blastocyst, it is apparent that the mouse blastocyst has many more high-affinity receptors than those in the mouse brain (Kd: 1.8 nM and Bmax: 18.8 pmol/mg membrane protein). Cannabinoid agonists and the CB1-R antagonist SR141716A effectively competed for anandamide binding in the blastocyst. To determine whether cannabinoid inhibition of embryonic development could be reversed by SR141716A, 2-cell embryos were cultured in the presence of cannabinoid agonists with or without SR141716A for 72 h. Most of the 2-cell embryos cultured in the absence of the agonists developed into blastocysts (approximately 90%). In contrast, the addition of cannabinoid agonists anandamide, Win 55212-2, or CP 55,940 in the culture medium severely compromised embryonic development: more than 60% of the 2-cell embryos failed to develop to blastocysts. A reduction in trophectoderm cell numbers was noted in those blastocysts that escaped the developmental arrest in the presence of cannabinoid agonists. However, this reduction was corrected when embryos were cultured simultaneously with an agonist and SR141716A. Furthermore, embryonic arrest was reversed when embryos were cultured simultaneously in the presence of an agonist and SR141716A. The addition of SR141716A alone in the culture medium apparently had no effects on embryonic development: more than 90% of the embryos developed into

blastocysts. The results suggest that the CB1 receptors in preimplantation mouse embryos are biologically active and cannabinoid effects on them are primarily mediated by these receptors.

Yao J, Milliez J, Netter A, Roux C, Reznikoff-Etievant MF. **[Embryotoxic factors and recurrent spontaneous abortions]**. J Gynecol Obstet Biol Reprod (Paris) 1997;26(3):304-8. (Fre)
About 30% of recurrent spontaneous abortions remain unexplained by traditional or biological anomalies. The purpose of this work was to investigate embryotoxic factors produced by trophoblast stimulated lymphocytes from women with unexplained recurrent spontaneous abortion. The samples from 36 women with recurrent abortion before and during the next pregnancy and from 7 women with normal pregnancies and no history of spontaneous abortion have been tested. The lymphocytes were stimulated with trophoblastic extracts. The supernatants of the stimulated lymphocytes were tested in a 4-cell mouse embryo culture. The secretion of embryotoxic factor was determined if the number of life blastocysts was less than 50% of control values after 4 days. The lymphocytes from 59% women with 3 or more recurrent abortion produced the embryotoxic factor, this factor may be useful in predicting pregnancy outcome in women with a history of unexplained recurrent miscarriage. The embryotoxic factor might be a new cause of recurrent abortion and a predictive factor.

Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. **From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells.** Proc Natl Acad Sci U S A 1999;96(10):5458-63.
Overexpression of the HER2/Neu protooncogene has been linked to the progression of breast cancer. Here we demonstrate that the growth of prostate cancer LNCaP cells can also be increased by the stable transfection of HER2/Neu. Using AG879, a HER2/Neu inhibitor, and PD98059, a MAP kinase inhibitor, as well as MAP kinase phosphatase-1 (MPK-1), in the transfection assay, we found that HER2/Neu could induce prostate-specific antigen (PSA), a marker for the progression of prostate cancer, through the MAP kinase pathway at a low androgen level. Reporter assays and mammalian two-hybrid assays further suggest this HER2/Neu-induced androgen receptor (AR) transactivation may function through the promotion of interaction between AR and AR coactivators, such as ARA70. Furthermore, we found this HER2/Neu --> MAP kinase --> AR-ARAs --> PSA pathway could not be blocked completely by hydroxyflutamide, an antiandrogen used in the treatment of prostate cancer. Together, these data provide a novel pathway from HER2/Neu to AR transactivation, and they may represent one of the reasons for the PSA re-elevation and hormone resistance during androgen ablation therapy in prostate cancer patients.

Young JF, Branham WS, Sheehan DM, Baker ME, Wosilait WD, Luecke RH. **Physiological "constants" for PBPK models for pregnancy.** J Toxicol Environ Health 1997 Dec 12;52(5):385-401.
Physiologically based pharmacokinetic (PBPK) models for pregnancy are inherently more complex than conventional PBPK models due to the growth of the maternal and embryo/fetal tissues. Physiological parameters such as compartmental volumes or flow rates are relatively constant at any particular time during gestation when an acute experiment might be conducted, but vary greatly throughout the course of gestation (e.g., contrast relative fetal weight during the first month of gestation with the ninth month). Maternal physiological parameters change during gestation, depending upon the particular system; for example, cardiac output increases by approximately 50% during human gestation; plasma protein concentration decreases during pregnancy; overall metabolism remains fairly constant. Maternal

compartmental volumes may change by 10-30%; embryo/fetal volume increases over a billionfold from conception to birth. Data describing these physiological changes in the human are available from the literature. Human embryo/fetal growth can be well described using the Gompertz equation. By contrast, very little of these same types of data is available for the laboratory animal. In the rodent there is a dearth of information during organogenesis as to embryo weights, and even less organ or tissue weight or volume data during embryonic or fetal periods. Allometric modeling offers a reasonable choice to extrapolate (approximately) from humans to animals; validation, however, is confined to comparisons with limited data during the late embryonic and fetal periods of development (after gestation d 11 in the rat and mouse). Embryonic weight measurements are limited by the small size of the embryo and the current state of technology. However, the application of the laser scanning confocal microscope (LSCM) to optically section intact embryos offers the capability of precise structural measurements and computer-generated three-dimensional reconstruction of early embryos. Application of these PBPK models of pregnancy in laboratory animal models at teratogenically sensitive periods of development provides exposure values at specific target tissues. These exposures provide fundamentally important data to help design and interpret molecular probe investigations into mechanisms of teratogenesis.

Yuan XJ, McCarthy BD, Salgar SK, Kunz HW, Gill TJ 3rd. **Biological effects of genes in the Grc and EC region of the rat major histocompatibility complex.** Am J Reprod Immunol 1999;42(1):64-9.
PROBLEM: To study the mechanism of action of major histocompatibility complex (MHC)-linked genes affecting reproduction, growth, and susceptibility to chemical carcinogens. METHOD OF STUDY: Tumors derived from rat embryonic fibroblasts were transfected with cosmids from the Grc and its linked regions, the unrelated A region, and a nonMHC region, or with genes from the Grc, Grc-linked, and nonMHC regions, to determine whether they could suppress tumor growth as determined by in vitro (soft agar) and in vivo assays. RESULTS: Tumor fibroblasts transfected with cosmids from the Grc or from the EC region decreased tumor growth in both the in vitro and in vivo assays. Transfection with individual genes from the Grc had no effect on tumor growth in either assay. CONCLUSIONS: The effects of the Grc on reproduction, growth, and tumorigenesis are mediated by extended genetic effects, i.e., by the conformation of the DNA in this region. Similar effects were seen following transfection with cosmids from the Grc-linked EC region, and this finding strengthens the hypothesis that the conformation of the DNA in this general region is critical for its function. A similar effect has been described for the locus control region (LCR) in the beta-globin gene family in the human.

MISCELLANEOUS

Benko I, Hernadi F, Megyeri A, Kiss A, Somogyi G, Tegyey Z, Kraicsovits F, Kovacs P. **Comparison of the toxicity of fluconazole and other azole antifungal drugs to murine and human granulocyte-macrophage progenitor cells in vitro.** J Antimicrob Chemother 1999;43(5):675-81.
We studied the inhibitory effects on colony formation by granulocyte-macrophage colony forming units (cfu-gm) of eight azole antifungal agents in vitro. All agents, except fluconazole, inhibited colony formation dose-dependently with 50% inhibitory concentrations (IC50) in the range of 0.78-49 micromol/L in cultures of murine and human bone marrow. For human cells, the IC50 values were 0.553 mg/L for itraconazole, 1.24 mg/L for saperconazole, 2.58 mg/L for clotrimazole, 5.33 mg/L for

miconazole, 6.17 mg/L for econazole, 6.27 mg/L for ketoconazole and 8.38 mg/L for oxiconazole. The IC50 of itraconazole for human cfu-gm in vitro was similar to the plasma level of this drug recommended for systemic antifungal therapy (>0.5 mg/L) thus indicating the potential clinical relevance of our data. The IC50 of ketoconazole for human cfu-gm in vitro may be exceeded by plasma levels produced in vivo by high (> or =400 mg) doses, whereas fluconazole failed to reduce colony formation by 50% even at 100 mg/L, a concentration not reached in vivo even after extremely high doses (2000 mg/day). To most of the drugs studied, murine progenitor cells seemed to be less sensitive than the human ones. There was, however, a close correlation between the murine and human log IC50 values of the drugs (r2 = 0.964, P< 0.001), suggesting that cultures of murine bone marrow may be suitable to predict the in-vitro toxicity of azole antifungals to human cfu-gm.

James JA, Sayers NM, Drucker DB, Hull PS. **Effects of tobacco products on the attachment and growth of periodontal ligament fibroblasts.** J Periodontol 1999;70(5):518-25.
BACKGROUND: Cigarette smoking is one of the most significant risk factors in the development and further advancement of inflammatory periodontal disease, however, the role of either nicotine or its primary metabolite cotinine in the progression of periodontitis is unclear. This study aimed to investigate the effects of nicotine and cotinine on the attachment and growth of fibroblasts derived from human periodontal ligament (PDL). METHODS: Primary cultures were prepared from the roots of extracted premolar teeth. Cells were used at both low (P3 to P5) and high (P11 to P13) passage. Cell numbers were determined over 14 days using either the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay or with a Coulter counter. Cultures were exposed to culture medium supplemented with 1) 15% fetal calf serum (FCS) only; 2) 1% FCS only; 3) 1% FCS and nicotine (concentration range 5 ng/ml to 10 mg/ml); or 4) 1% FCS and cotinine (concentration range 0.5 ng/ml to 10 microg/ml). RESULTS: Nicotine significantly (P <0.05, by ANOVA) inhibits attachment and growth of low passage cells at concentrations >1 mg/ml and high passage PDL fibroblasts at concentrations >0.5 mg/ml. Cotinine, at the highest concentration used (10 microg/ml), appeared to inhibit attachment and growth of both low and high passage fibroblasts but this was not statistically significant (P >0.05, by ANOVA). CONCLUSIONS: Tobacco products inhibit attachment and growth of human PDL fibroblasts. This may partly explain the role of these substances in the progression of periodontitis.

Pohl HR, Roney N, Fay M, Chou CH, Wilbur S, Holler J. **Site-specific consultation for a chemical mixture.** Toxicol Ind Health 1999;15(5):470-9.
The Agency for Toxic Substances and Disease Registry (ATSDR) uses the weight of evidence methodology to evaluate interactions of chemical mixtures. In the process, toxicity, toxicokinetics, and toxicodynamics of chemical components of the mixture are carefully examined. Based on the evaluation, predictions are made that can be used in real-life situations at hazardous waste sites. In this paper, health outcomes were evaluated for a mixture of eight compounds that were found at a specific site. These eight chemicals were identified and possibly associated with human exposure. The health assessors could consider similar thought processes when evaluating chemical mixtures at hazardous waste sites.

Schlage WK, Buelles H, Kurkowsky B. **Use of the HET-CAM test for the determination of the irritant potential of cigarette sidestream smoke.** Toxicol In Vitro 1999;13(4-5):829-35.
BIOSIS COPYRIGHT: BIOL ABS. We investigated the suitability of the HET-CAM test for the determination of the irritant potential of cigarette sidestream smoke (SS). Coded test solution (0.2 ml)

was applied to the chorioallantoic membrane (CAM) of 10 day-old White Leghorn chick embryos. The time from the start of exposure to the occurrence of blood vessel injection (I), haemorrhage (H) and coagulation (C) in the first 5 minutes was recorded, and irritancy scores (IS) from 0 to 21 were calculated (Luepke, 1985). For the micro ited IS from 3 to 7.5, an underestimation due to mortality associated with an immediate breakdown ofblood circulation through the CAM vessels, thereby interfering with the irritation parameters I and H. We propose to test blood circulation-independent irritation endpoints, for example trypan blue uptake in the CAM (INVITTOX/ERGATT/FRAME, 1996), for determination of the irritancy of SS.

Serabian MA, Pilaro AM. **Safety assessment of biotechnology-derived pharmaceuticals: ICH and beyond.** Toxicol Pathol 1999;27(1):27-31.
Many scientific discussions, especially in the past 8 yr, have focused on definition of criteria for the optimal assessment of the preclinical toxicity of pharmaceuticals. With the current overlap of responsibility among centers within the Food and Drug Administration (FDA), uniformity of testing standards, when appropriate, would be desirable. These discussions have extended beyond the boundaries of the FDA and have culminated in the acceptance of formalized, internationally recognized guidances. The work of the International Committee on Harmonisation (ICH) and the initiatives developed by the FDA are important because they (a) represent a consensus scientific opinion, (b) promote consistency, (c) improve the quality of the studies performed, (d) assist the public sector in determining what may be generally acceptable to prepare product development plans, and (e) provide guidance for the sponsors in the design of preclinical toxicity studies. Disadvantages associated with such initiatives include (a) the establishment of a historical database that is difficult to relinquish, (b) the promotion of a check-the-box approach, i.e., a tendancy to perform only the minimum evaluation required by the guidelines, (c) the creation of a disincentive for industry to develop and validate new models, and (d) the creation of state-of-the-art guidances that may not allow for appropriate evaluation of novel therapies. The introduction of biotechnology-derived pharmaceuticals for clinical use has often required the application of unique approaches to assessing their safety in preclinical studies. There is much diversity among these products, which include the gene and cellular therapies, monoclonal antibodies, human-derived recombinant regulatory proteins, blood products, and vaccines. For many of the biological therapies, there will be unique product issues that may require specific modifications to protocol design and may raise additional safety concerns (e.g., immunogenicity). Guidances concerning the design of preclinical studies for such therapies are generally based on the clinical indication. Risk versus benefit decisions are made with an understanding of the nature of the patient population, the severity of disease, and the availability of alternative therapies. Key components of protocol design for preclinical studies addressing the risks of these agents include (a) a safe starting dose in humans, (b) identification of potential target organs, (c) identification of clinical parameters that should be monitored in humans, and (d) identification of at-risk populations. One of the distinct aspects of the safety evaluation of biotechnology-derived pharmaceuticals is the use of relevant and often nontraditional species and the use of animal models of disease in preclinical safety evaluation. Extensive contributions were made by the Center for Biologics Evaluation and Research to the ICH document on the safety of biotherapeutics, which is intended to provide worldwide guidance for a framework approach to the design and review of preclinical programs. Rational, scientifically sound study design and early identification of the potential safety concerns that may be anticipated in the clinical trial can result in

preclinical data that facilitate use of these novel therapies for use in humans without duplication of effort or the unnecessary use of animals.

Van Landuyt P, Peter B, Beluze L, Lemaitre J. **Reinforcement of osteosynthesis screws with brushite cement.** Bone 1999;25(2 Suppl):95s-98s.
The fixation of osteosynthesis screws remains a severe problem for fracture repair among osteoporotic patients. Polymethyl-methacrylate (PMMA) is routinely used to improve screw fixation, but this material has well-known drawbacks such as monomer toxicity, exothermic polymerization, and nonresorbability. Calcium phosphate cements have been developed for several years. Among these new bone substitution materials, brushite cements have the advantage of being injectable and resorbable. The aim of this study is to assess the reinforcement of osteosynthesis screws with brushite cement. Polyurethane foams, whose density is close to that of cancellous bone, were used as bone model. A hole was tapped in a foam sample, then brushite cement was injected. Trabecular osteosynthesis screws were inserted. After 24 h of aging in water, the stripping force was measured by a pull-out test. Screws (4.0 and 6.5 mm diameter) and two foam densities (0.14 and 0.28 g/cm3) were compared. Cements with varying solid/liquid ratios and xanthan contents were used in order to obtain the best screw reinforcement. During the pull-out test, the stripping force first increases to a maximum, then drops to a steady-state value until complete screw extraction. Both maximum force and plateau value increase drastically in the presence of cement. The highest stripping force is observed for 6.5-mm screws reinforced with cement in low-density foams. In this case, the stripping force is multiplied by 3.3 in the presence of cement. In a second experiment, cements with solid/liquid ratio ranging from 2.0 to 3.5 g/mL were used with 6.5-mm diameter screws. In some compositions, xanthan was added to improve injectability. The best results were obtained with 2.5 g/mL cement containing xanthan and with 3.0 g/mL cements without xanthan. A 0.9-kN maximal stripping force was observed with nonreinforced screws, while 1.9 kN was reached with reinforced screws. These first results are very promising regarding screw reinforcement with brushite cement. However, the polyurethane foam model presents noninterconnected porosity and physiological liquid was not modelized.

Whaley DA, Meloy TP, Barrett SS, Bedillion EJ. **Incorporation of potential for multimedia exposure into chemical hazard scores for pollution prevention.** Drug Chem Toxicol 1999;22(1):241-73.
We are reporting a chemical hazard score for pollution prevention, called the Purdue score. The Purdue score provides a relative quantitative measure combining a variety of chemical hazards into a single quantitative hazard weighting factor for the non-expert to use. The main expected uses are to design safer products, assist in implementing and measuring achievement in pollution prevention, and as an adjunct for reporting Toxic Release Inventory data to the U.S. Government. Scoring results are presented for 200 Superfund chemicals, rank ordered by the worker hazard part of the score, by the environmental hazard part, and by combined worker and environmental hazard scores. We have reviewed the extent to which the Purdue score presently incorporates potential for multimedia pathway and multiroute absorption exposure. Until other possible uses have been carefully tested, peer-reviewed and published, users are advised to limit use of this system to planning, implementing and measuring pollution prevention and to enhancing the interpretation of Toxic Release Inventory data. The objective of this report is to look at how the structure of this score handles exposure to chemicals, both via multi-compartment pathways and multi-routes for contact or absorption health damage, as well as how it

handles habitat degradation by chemicals. For all of these, the approach is built on inherent properties of each chemical, which are true for all sites and scenarios. The biggest obstacle to scoring is lack of measured chemical property data needed for scoring. We handle missing data by regression, quantitative structure activity relationship estimations, and a missing data default rule. The limitations of chemical hazard scoring are reviewed. At present, there is no widely accepted single measure of relative chemical hazard, against which to calibrate this hazard score for accuracy, except experience from industrial use. However, despite limitations, we suggest there is a strong value added for industry and society in availability of a concise, simple-to-use measure of relative chemical hazard. The Purdue score enables separate or combined consideration of chemical hazard to workers and to the natural environment. The Purdue score has potential for major cost savings in relative hazard ranking and business decision making regarding little-studied organic chemicals, because of the extensive use of advanced property estimation software. We conclude that there is societal need to warrant advanced development of this risk management tool, which is now ready for pilot use by industry. The Purdue score is mainly intended to assist and encourage businesses to implement and measure pollution prevention-especially small businesses--in a cost-effective way. The Purdue score relies strongly on sublethal toxicity, and there is practical potential for it to be used with thousands of chemicals.